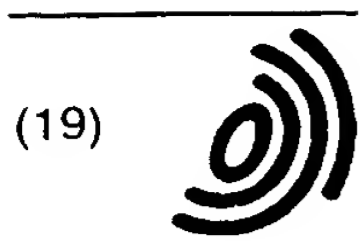


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(54) **Osteoclastgenic inhibitory agent comprising interleukin-18**

(57) An osteoclastgenic inhibitory agent which comprises an interleukin-18 and/or its functional equivalent. The agent can be arbitrarily used as an ingredient for

cell culture and agents for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

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## Description

The present invention relates to an osteoclastgenic inhibitory agent comprising an interleukin-18 (hereinafter abbreviated as "IL-18") or its functional equivalent.

Osteoblasts' bone formation and osteoclasts' bone resorption are well balanced in healthy living bodies, and this keeps the bone tissues in normal conditions while old bone tissues are being replaced with fresh ones without altering the original bone shape. The phenomenon plays an important role in keeping living bodies' homeostasis such as the controlling of blood calcium concentration within a desired range. Once the balance is lost, especially when the bone resorption level exceeds the bone formation level, bone-related diseases and other diseases may be induced. Therefore, elucidation of the whole mechanism of bone resorption in living bodies, particularly, elucidation of osteoclasts is greatly highlighted due to scientific and clinical significance thereof.

However, the mechanism of osteoclast formation has not yet been completely elucidated even though interleukin 1 as a promoter and interleukin 4 as an inhibitor were found. This is because, similarly as various phenomena in living bodies, osteoclast formation in living bodies is controlled by the close and complicated relationship between promoters and inhibitors. Based on these, it is greatly expected to establish an effective osteoclastgenic inhibitory agent from the viewpoint of scientific and clinical aspects.

The object of the present invention is to provide a novel and effective osteoclastgenic inhibitory agent. To solve the object the present inventors energetically studied for IL-18, i.e., one of cytokines as communication transferring substances in immune systems, which induces production of interferon- $\gamma$  (hereinafter abbreviated as "IFN- $\gamma$ "), an important biologically active substance for immunocompetent cells, and granulocyte/macrophage colony-stimulating factor (hereinafter abbreviated as "GM-CSF"), and augments cytotoxicity and induces formation of killer cells. At the finding, IL-18 was described as an **interferon- $\gamma$ -inducing factor** as reported by Haruki OKAMURA in Japanese Patent Kokai Nos. 27,189/96 and 193,098/96, and in *Nature*, Vol. 378, No. 6,552, pp. 88-91 (1995), and then called IL-18 according to the proposal of Shimpei USHIO et al., in *The Journal of Immunology*, Vol. 156, pp. 4,274-4,279 (1996).

The present inventors found that a particular gene, capable of inhibiting osteoclast formation from osteoclastic precursor cells *in vitro*, is specifically expressed in quantities in stroma cells derived from mouse myeloma. Their further detailed analysis revealed that (i) the gene encodes IL-18 that includes SEQ ID NO: 7 as a core sequence, (ii) IL-18 and functional equivalents thereof effectively inhibit osteoclast formation, and (iii) the inhibition is mainly due to the action of GM-CSF induced and produced by IL-18.

Based on these, the present inventors solved the present object by an osteoclastgenic inhibitory agent comprising IL-18 or its functional equivalent as an effective ingredient.

FIG. 1 shows the structure of the recombinant DNA pKGFHH2.

FIG. 2 shows the structure of the recombinant DNA pCSHIGIF/MUT35.

FIG. 3 shows the structure of the recombinant DNA pCSHIGIF/MUT42.

FIG. 4 shows the structure of the recombinant DNA pBGHuGF.

FIG. 5 shows the structure of the recombinant DNA pKGFMH2.

In these figures, KGFHH2 cDNA means a cDNA encoding the IL-18 according to the present invention; IGIF/MUT35: a DNA encoding the IL-18 according to the present invention; IGIF/MUT42: a DNA encoding the IL-18 according to the present invention; HuIGIF: a chromosomal DNA encoding the IL-18 according to the present invention; KGFMH2 cDNA: a cDNA encoding the IL-18 according to the present invention; 5S: a gene for 5S ribosomal RNA; P<sub>tac</sub>: a tac promoter; rrnBT1T2: a termination region of a ribosomal RNA operon; Amp<sup>R</sup>: an ampicillin resistant gene; pBR322ori: a replication origin of *Escherichia coli*; CMV: a cytomegalovirus promoter; IFNss: a nucleotide sequence encoding a signal peptide for subtype  $\alpha 2b$  of human interferon- $\alpha$ .

The present invention relates to an osteoclastgenic inhibitory agent comprising IL-18 or its functional equivalent as an effective ingredient. The wording "IL-18" as referred to in the invention includes polypeptides with the above property independently of their sources and origins. For example, the IL-18 used in the present invention includes, as internal partial amino acid sequences, the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3, as well as SEQ ID NO: 4 and SEQ ID NO: 5, and includes the amino acid sequence of SEQ ID NO: 6 or SEQ ID NO: 7 as a whole. The wording "**functional equivalent(s)**" as referred to in the present invention includes (i) those wherein one or more amino acids in the amino acid sequence of IL-18 are replaced with different amino acids, (ii) those wherein one or more amino acids are added to the N- and/or C-termini of the amino acid sequence of IL-18, (iii) those wherein one or more amino acids are inserted into the internal sites of the amino acid sequence of IL-18, (iv) those wherein one or more amino acids in the N- and/or C-terminal regions of the amino acid sequence of IL-18 are deleted, and (v) those wherein one or more amino acids in the internal regions of the amino acid sequence of IL-18 are deleted; all of these modifications should be made within the range that does not substantially lose the property of osteoclast formation by IL-18 among the inherent property of IL-18. Examples of such functional equivalents are described along with their detailed amino acid sequences in Japanese Patent Application No. 20,906/97 by the same applicant of the present applicant, i.e., polypeptides which are capable of inducing production of interferon-gamma by immunocompe-

tent cells, wherein said polypeptides contain either amino acid sequence wherein one or more cysteines are replaced with different amino acid(s) while leaving respective consensus sequences as shown in SEQ ID NOs: 1, 2 and 4 intact, or that wherein one or more amino acids are added, removed and/or replaced at one or more sites including those in the consensus sequences but excluding those of the replaced cysteine. The different amino acids to replace the cysteine(s) are not restricted to any types, as far as the resulting polypeptide, containing an amino acid sequence replaced with the different amino acid(s), exhibits an activity of inducing production of IFN- $\gamma$  by immunocompetent cells in the presence or absence of an appropriate cofactor, as the wild-type polypeptides containing SEQ ID NOs: 1, 2 and 4 as consensus partial amino acid sequences, and a stability significantly higher than that of the wild-type polypeptides. The different amino acids include serine, threonine, alanine, valine, leucine, isoleucine, histidine, tyrosine, phenylalanine, tryptophan, and methionine, among which the most preferable amino acid is serine or alanine. Embodiments of the amino acid sequences, containing SEQ ID NOs: 1, 2 and 4 as consensus partial amino acid sequences, in which one or more cysteines are to be replaced with different amino acid(s) are the wild-type polypeptides containing SEQ ID NO: 6 or 7. SEQ ID NO: 6 contains cysteines at the 38th, 68th, 76th, and 127th positions from the N-terminus. SEQ ID NO: 7 contains cysteines at the 7th, 75th, and 125th positions. The polypeptides include those containing the amino acid sequence of any one of SEQ ID NOs: 20-26, which are derived from the wild-type polypeptide containing SEQ ID NO: 6, those containing the amino acid sequence of SEQ ID NO: 27 or 28, which are derived from the wild-type polypeptide containing the amino acid sequence of SEQ ID NO: 7, and those containing an amino acid sequence derived from any one of SEQ ID NOs: 20-28 by adding, removing, and/or replacing one or more amino acids to and/or at position(s) excepting the positions where the cysteine(s) have been replaced while retaining the desired biological activities and stability. The wording "one or more amino acids" means the number of amino acids which conventional methods such as site-directed mutagenesis can usually add, remove or replace. The polypeptides containing any one of SEQ ID NOs: 20-28 possess both stability and biological activities significantly higher than those of the wild-type polypeptides.

The functional equivalents as referred to in the present invention further include glycosylated polypeptides of IL-18 and the above polypeptides. Any of these IL-18 and functional equivalents thereof, both of which are included to and referred to as "IL-18" in the present invention, unless specified otherwise, can be used in the present invention independently of their origins; those prepared by separating from natural sources such as cell cultures and from artificially synthesized ones using recombinant DNA technology and peptide synthesis.

With economical viewpoint, methods of recombinant DNA technology are advantageously used: generally, desired IL-18 can be obtained by introducing DNAs encoding IL-18 into appropriate hosts derived from microorganisms, plants, and animals to form transformants, culturing the transformants in nutrient culture media in a conventional manner, and purifying the cultures by conventional methods used for purifying cytokines. Any DNAs can be used as the above DNAs as long as they contain a DNA encoding IL-18, and can be suitably selected depending on the purpose of the use of the present osteoclastgenic inhibitory agent or on the recombinant DNA technology used. For example, Japanese Patent Kokai Nos. 193,098/96, 231,598/96, and 27,189/96 by the same applicant of the present invention disclose in detail methods for producing IL-18 by culturing transformed microorganisms into which DNAs including a cDNA encoding mouse or human IL-18 are introduced; and Japanese Patent Application No. 185,305/96 by the same applicant of the present invention discloses in detail a method for producing IL-18 encoding human IL-18 by culturing transformed animal cells which have an introduced DNA that includes a chromosomal DNA encodes human IL-18. Japanese Patent Application No. 20,906/97 by the same applicant of the present invention discloses in detail a method for producing IL-18 by culturing transformed animal cells having an introduced DNA which includes a DNA encoding a functional equivalent of human IL-18.

The aforesaid recombinant DNA technology has an economical advantage, but depending on the hosts and DNA sequences used, the IL-18 thus obtained may have somewhat different physicochemical property from those of IL-18 produced and functions *in vivo*. Japanese Patent Application No. 67,434/96 by the same applicant of the present invention discloses in detail a preparation of IL-18 using established human cell lines as natural sources, and Japanese Patent Application No. 213,267/96 by the same applicant also discloses in detail the preparation using an interleukin-1 $\beta$ -converting enzyme. The IL-18 obtained by those preparations can be estimated to have substantially the same or equal physicochemical property to that of IL-18 that is produced and functions *in vivo*, and the yield can be estimated to be slightly lower. However, such IL-18 has an advantage that it has a fewer side effects when used as pharmaceuticals directed to administering to warm-blooded animals in general and including humans. When applying purification methods using monoclonal antibodies specific to IL-18, as disclosed in Japanese Patent Application No. 231,598/96 by the same applicant of the present invention, a relatively-high purity IL-18 can be obtained in a minimum labor and cost.

The present osteoclastgenic inhibitory agent comprising the aforesaid IL-18 includes any types and forms usable to inhibit osteoclast formation both *in vivo* and *in vitro*. The present agent can be advantageously used as ingredients for cell culture media for animal cells, which satisfactorily inhibit osteoclast formation, maintain, proliferate, and/or differentiate the desired cells; components of screening kits for bone-related therapeutic agents; bone-resorption regulatory agents; and agents for osteoclast-related diseases. The bone-resorption regulatory agents include medica-

ments and health foods that exert an osteoclastgenic inhibitory activity *in vivo*, control bone resorption to normal conditions, and improve unfavorable physical conditions such as a relatively-insignificant arthralgia. The agents for osteoclast-related diseases include medicaments used to prevent and/or treat diseases caused by an excessive osteoclast formation and/or its function. Examples of such diseases are hypercalcemia, osteoclastoma, Behcet's syndrome, osteosarcoma, arthropathy, chronic rheumatoid arthritis, deformity osteitis, primary hyperthyroidism, osteopenia, and osteoporosis. Varying depending on the types of agents and diseases to be treated, the present agent is usually formulated into a liquid, paste, or solid form which contains 0.000002-100 w/w %, preferably, 0.0002-0.5 w/w % of IL-18.

The present osteoclastgenic inhibitory agent can be IL-18 alone or compositions comprising IL-18 and one or more other ingredients such as carriers, excipients, diluents, adjuvants, antibiotics, and proteins such as serum albumin and gelatin as stabilizers; saccharides such as glucose, maltose, maltotriose, maltotetraose, trehalose, sucrose, isomaltose, lactose, panose, erlose, palatinose, lactosucrose, raffinose, fructooligosaccharide, galactooligosaccharide, lentinan, dextrin, pullulan, and sugar alcohols including sorbitol, maltitol, lactitol, and maltotriitol; buffers comprising phosphates or citrates mainly; and reductants such as 2-mercaptoethanol, dithiothreitol, and reduced glutathione; and optionally biologically active substances such as interferon- $\alpha$ , interferon- $\beta$ , interferon- $\gamma$ , interleukin-2, interleukin-3, interleukin-6, interleukin-12, TNF- $\alpha$ , TNF- $\beta$ , GM-CSF, estrogen, progesterone, chlormadinone acetate, calcitonin, somatostatin, somatomedin, insulin-like growth factor, ipriflavone, parathyroid hormone (PTH), norethisterone, busulfan, acyclovir, cytarabine, fluorouracil, tetrahydrofurfuryl fluorouracil, methotrexate, vitamin D<sub>2</sub>, active vitamin D, Krestin® or polysaccharide K, L-asparaginase, and OK-432 or Picibanil®; and calcium salts such as calcium lactate, calcium chloride, calcium monohydrogenphosphate, and L-calcium L-aspartate. When used as agents for administering to warm-blooded animals in general and including humans, i.e., agents for osteoclast-related diseases, the present agent can be preferably formulated into compositions by appropriately combining with one or more of the above physiologically-acceptable substances.

The present osteoclastgenic inhibitory agent includes medicaments in a unit dose form used for administering to warm-blooded animals in general and including humans. The wording "**unit dose form**" means those which contain IL-18 in an amount suitable for a daily dose or in an amount up to four fold by integers or up to 1/40 fold of the dose, and those in a physically separated and formulated form suitable for prescribed administrations. Examples of such formulations are injections, liquids, powders, granules, tablets, capsules, troches, collyriums, nebulas, and suppositories.

The present agent as an osteoclastgenic inhibitory agent effectively treat and prevent osteoclast-related diseases independently of oral and parenteral administrations. Varying depending on the types and symptoms of patients' diseases, the present agent can be administered to the patients orally, intradermally, subcutaneously, muscularly, or intravenously at a dose of about 0.5  $\mu$ g to 100 mg per shot, preferably, at a dose of about 2  $\mu$ g to 10 mg per shot of IL-18, 2-6 fold a day or 2-10 fold a week for one day to one year.

In the below, with reference to experiments, the preparation, physicochemical property, and biological activity of the IL-18 according to the present invention are described:

#### Experiment 1

##### Preparation of human IL-18

According to the method in Japanese Patent Kokai No. 231,598/96 by the same applicant of the present invention, an autonomously-replicable recombinant DNA, pKGFHH2, linked to a cDNA encoding human IL-18, was prepared. Dideoxyribonucleotide sequencing analyzed that, as shown in FIG. 1, in the recombinant DNA, KGFHH2 cDNA containing the base sequence of SEQ ID NO: 8 was linked to the downstream of Ptac, a Tac promoter. The recombinant DNA pKGFHH2 contained the amino acid sequences of SEQ ID NOs: 1 to 5; these amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 8.

According to the method in Japanese Patent Kokai No. 231,598/96, the recombinant DNA pKGFHH2 was introduced into an *Escherichia coli* Y1090 strain, ATCC 37197, and the strain was cultured. The produced polypeptide was purified by immunoaffinity chromatography to obtain a purified human IL-18 with a purity of at least 95% in a yield of about 25 mg/l culture. According to the method in Japanese Patent Kokai No. 193,098/96 by the same applicant of the present invention, the purified human IL-18 was analyzed for biological activity and physicochemical property as indicated below: When culturing human lymphocytes, collected by a conventional manner from a healthy donor, in the presence of the purified human IL-18, IFN- $\gamma$  production was observed depending on the concentration of IL-18, resulting in a confirmation that IL-18 has an activity of inducing IFN- $\gamma$  production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified IL-18 was subjected to SDS-PAGE, resulting in a major band with an IFN- $\gamma$  inducing activity at a position corresponding to 18,500 $\pm$ 3,000 daltons. The IL-18 gave a pI of 4.9 $\pm$ 1.0 as determined by conventional chromatofocusing. Conventional analysis using "PROTEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City,

USA, revealed that the IL-18 had the amino acid sequence of SEQ ID NO: 9, i.e., the amino acid sequence of SEQ ID NO: 8 where a methionine residue was linked to the N-terminus.

## Experiment 2

### Preparation of human IL-18

According to the method in Japanese Patent Application No. 67,434/96 by the same applicant of the present invention, THP-1 cells, ATCC TIB 202, a human monocyte cell line derived from a male with acute monocytic leukemia, were inoculated to the dorsum subcutaneous tissues of new born hamsters, followed by feeding the hamsters for three weeks. Tumor masses, about 15 g weight each, formed in the subcutaneous tissues of each hamster, were extracted, dispersed in media, and disrupted. The polypeptide obtained from the disrupted cells was purified by immunoaffinity chromatography to obtain a purified human IL-18 in a yield of an about 50 ng/head.

Similarly, according to the method in Japanese Patent Application No. 67,434/96, the purified human IL-18 was analyzed and determined for biological activity and physicochemical property as indicated below: It was confirmed that culturing human lymphocytes, collected from healthy donors in a conventional manner, in the presence of different concentrations of the human IL-18, resulted in an IL-18 dose-dependent IFN- $\gamma$  production. This revealed that the human IL-18 has a biological activity of inducing IFN- $\gamma$  production by lymphocytes as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE using 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN- $\gamma$  production inducing activity at a position corresponding to 18,000-19,500 daltons. According to the peptide map disclosed in Japanese Patent Application No. 67,434/96, the human IL-18 was treated with clostripain commercialized by Sigma Chemical Company, Missouri, USA, to obtain polypeptide fragments, followed by subjecting the fragments for fractionation to high-performance liquid chromatography (HPLC) using "ODS-120T", a column commercialized by Tosoh Corporation, Tokyo, Japan, and analyzing the amino acid sequences of the fragments from the N-terminus to reveal the following amino acid sequences of SEQ ID NOs: 10 to 13. These amino acid sequences were completely coincided with amino acids 148-157, 1-13, 45-58, and 80-96 in SEQ ID NO: 6. The data shows that the human IL-18 obtained in Experiment 2 has the amino acid sequence of SEQ ID NO: 6 and all the partial amino acid sequences of SEQ ID NOs: 1 to 5.

## Experiment 3

### Preparation of functional equivalents

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, it was prepared an autonomously-replicable recombinant DNA. pCSHIGIF/MUT35, was linked to a DNA encoding a functional equivalent of human IL-18 where cysteines 38, 68, and 76 in SEQ ID NO: 6 were respectively replaced with serine, serine, and alanine. Dideoxyribonucleotide sequence analysis revealed that as shown in FIG. 2, in the recombinant DNA. DNA IGIF/MUT35 with SEQ ID NO: 14 linked to the downstream of a base sequence encoding a signal peptide of subtype  $\alpha 2b$  in human interferon- $\alpha$  in the same reading-frame, as reported by K. Henco et al., in *Journal of Molecular Biology*, Vol. 185, pp. 227-260 (1985), and had a stop codon for protein synthesis at further downstream. As shown in parallel in SEQ ID NO: 14, the amino acid sequence encoded by the recombinant DNA corresponded to SEQ ID NO: 6 where cysteines 38, 68, and 76 in SEQ ID NO: 6 were respectively replaced with serine, serine, and alanine. The recombinant DNA contained a nucleotide which encodes all the amino acid sequences of SEQ ID NOs: 1 to 4 and the one of SEQ ID NO: 5 where cysteine at amino acid 5 in SEQ ID NO: 5 was replaced with alanine. These amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 14.

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, the recombinant DNA pCSHIGIF/MUT35 was introduced into COS-1 cells, ATCC CRL 1650, an established cell line derived from SV40 transformed African Green monkey kidney, followed by culturing the transformed cells. The produced polypeptide in the culture was purified by immunoaffinity chromatography to obtain a purified functional equivalent of human IL-18 in a yield of about 40 ng/ml culture. According to the method in Japanese Patent Application No. 20,906/97, the purified functional equivalent was analyzed and determined for biological activity and physicochemical property as indicated below: When culturing KG-1 cells, ATCC CCL 246, an established cell line derived from human acute myelogenous leukemia, in the presence of different concentrations of the purified functional equivalent of human IL-18, IFN- $\gamma$  production was observed depending on the concentration of the IL-18, revealing that the IL-18 has a biological activity of inducing IFN- $\gamma$  production by KG-1 cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified functional equivalent was

subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN- $\gamma$  production inducing activity at a position corresponding to 18,000-19,500 daltons. Conventional analysis using "PROTEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City, USA, revealed that the N-terminal region of the functional equivalent had the amino acid sequence of SEQ ID NO: 15 which corresponded to the amino acid sequence in the N-terminal region as shown in parallel in SEQ ID NO: 14.

#### Experiment 4

##### Preparation of functional equivalent

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, it was prepared an autonomously-replicable recombinant DNA, **pCSHIGIF/MUT42**, which was linked to a DNA encoding for a functional equivalent of human IL-18 where cysteines 38, 68, 76, and 127 in SEQ ID NO: 6 were respectively replaced with serine, serine, alanine, and serine. Dideoxyribonucleotide sequencing revealed that, as shown in FIG. 3, in the recombinant DNA, DNA IGIF/MUT42 with SEQ ID NO: 16 linked to the downstream of a base sequence encoding a signal peptide for subtype  $\alpha 2b$  of human interferon- $\alpha$  in the same reading frame, as reported by K. Henco et al., in *Journal of Molecular Biology*, Vol. 185, pp. 227-260 (1985), and had a stop codon for protein synthesis at further downstream. As shown in parallel in SEQ ID NO: 16, the amino acid sequence encoded by the recombinant DNA corresponded to SEQ ID NO: 6 where cysteines 38, 68, 76, and 127 in SEQ ID NO: 6 were respectively replaced with serine, serine, alanine, and serine. The recombinant DNA contained a nucleotide sequence which encodes all the amino acid sequences of SEQ ID NOs: 1 to 4 and the one of SEQ ID NO: 5 where cysteine 5 in SEQ ID NO: 5 was replaced with alanine. These amino acid sequences were respectively encoded by nucleotides 46-63, 88-105, 400-420, 151-165, and 214-228 in SEQ ID NO: 16.

According to the method in Japanese Patent Application No. 20,906/97 by the same applicant of the present invention, the recombinant DNA **pCSHIGIF/MUT42** was introduced into COS-1 cells, followed by culturing the cells. The produced polypeptide in the culture was purified by immunoaffinity chromatography to obtain a purified functional equivalent of human IL-18 in a yield of about 20 ng/ml culture. According to the method in Japanese Patent Application No. 20,906/97, the purified functional equivalent was analyzed and determined for biological activity and physicochemical property as indicated below: When cultured KG-1 cells in the presence of different concentrations of the purified functional equivalent, a dose-dependent IFN- $\gamma$  production was observed, and this revealed that the functional equivalent has a biological activity of inducing IFN- $\gamma$  production by KG-1 cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified functional equivalent was subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN- $\gamma$  inducing activity at a position corresponding to 18,000-19,500 daltons. Conventional analysis using "PROTEIN SEQUENCER MODEL 473A", an apparatus of Applied Biosystems, Inc., Foster City, USA, revealed that the N-terminal region of the functional equivalent had the amino acid sequence of SEQ ID NO: 15 which completely corresponded to the amino acid sequence in the N-terminal region as shown in parallel in SEQ ID NO: 16.

#### Experiment 5

##### Preparation of human IL-18

According to the method in Japanese Patent Application No. 185,305/96 by the same applicant of the present invention, an autonomously-replicable recombinant DNA, **pBGHuGF**, linked to a chromosomal DNA encoding human IL-18, was obtained. Dideoxyribonucleotide sequencing analysis revealed that as shown in FIG. 4, in the recombinant DNA, a chromosomal DNA, which encodes human IL-18, i.e., DNA HuIGIF with SEQ ID NO: 17, was linked to the downstream of a restriction site by a restriction enzyme, *Hind* III. As shown in SEQ ID NO: 17, the chromosomal DNA HuIGIF consists of 11,464 bp where the exon was fragmented by four introns positioning at nucleotides 83-1,453, 1,466-4,848, 4,984-6,317, and 6,452-11,224. Among the resting nucleotide sequence excluding these introns, nucleotides 3-11,443 from the 5'-terminus are the part that encodes a precursor of human IL-18, and nucleotides 4,866-4,983 are the part that encodes an active human IL-18. The chromosomal DNA contained nucleotide sequences encoding SEQ ID NOs: 1 to 5; these amino acid sequences were respectively encoded by nucleotides 4,911-4,928, 4,953-4,970, 11,372-11,392, 6,350-6,364, and 6,413-6,427 in SEQ ID NO: 17.

According to the method in Japanese Patent Application No. 185,305/96, the recombinant DNA **pBGHuGF** was introduced into CHO-K1 cells, ATCC CCL 61, an established cell line derived from Chinese hamster ovary, followed by culturing the cells. The culture supernatant was contacted with a supernatant of cell disruptant prepared from a THP-1 cell culture to produce a polypeptide which was then purified by immunoaffinity chromatography to obtain a purified human IL-18 in a yield of about 15 mg/l culture. According to the method in Japanese Patent Application No.

185,305/96, the polypeptide was analyzed and determined for biological activity and physicochemical property as indicated below: It was confirmed that human lymphocytes, which were collected from a healthy donor, produced IFN- $\gamma$  depending on the purified human IL-18 concentration when cultured at different concentrations of the human IL-18, revealing that the human IL-18 has a biological activity of inducing IFN- $\gamma$  production by lymphocytes as an immuno-competent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE in the presence of 2 w/v % dithiothreitol as a reductant, resulting in a major band with an IFN- $\gamma$  inducing activity at a position corresponding to 18,000-19,500 daltons. The N-terminal region of the human IL-18 contained the amino acid sequence of SEQ ID NO: 15 which completely corresponded to the amino acid sequence in the N-terminal region of SEQ ID NO: 17 for an active IL-18. Experiment 6

#### Preparation of mouse IL-18

To a 0.5-ml reaction tube were added 8  $\mu$ l of 25 mM magnesium chloride, 10  $\mu$ l of 10 x PCR buffer, one  $\mu$ l of 25 mM dNTP mix, one  $\mu$ l of 2.5 units/ $\mu$ l of amplitaq DNA polymerase, one ng of a recombinant DNA, which encodes mouse IL-18 having the nucleotide sequence of SEQ ID NO: 18 and the amino acid sequence of SEQ ID NO: 7, prepared from a phage DNA clone according to the method in Japanese Patent Kokai No. 27,189/96, and adequate amounts of a sense and antisense primers having nucleotide sequences represented by 5'-ATAGAATTCAAAT-GAACTTTGGCCGACTTCACTG-3' and 5'-ATAAAGCTTCTAACTTTGATGTAAGTT-3', respectively, which were chemically synthesized based on the amino acid sequences nearness to the N- and C-termini of SEQ ID NO: 7, and the mixture solution was brought up to a volume of 100  $\mu$ l with sterilized distilled water. The solution thus obtained was subjected in a usual manner to PCR reaction of the following three cycles of successive incubations at 94°C for one minute, 43°C for one minute, and 72°C for one minute, and further 40 cycles of successive incubations at 94°C for one minute, 60°C for one minute, and 72°C for one minute.

The product obtained by the PCR reaction and "pCR-Script SK (+)", a plasmid vector commercialized by Stratagene Cloning Systems, California, USA, were in a conventional manner ligated together using a DNA ligase into a recombinant DNA which was then introduced into "XL-1 Blue MRF<sup>+</sup>Kan", an *Escherichia coli* strain commercialized by Stratagene Cloning Systems, California, USA, to obtain a transformant. The transformant was inoculated to L-broth (pH 7.2) containing 50  $\mu$ g/ml ampicillin, followed by the incubation at 37°C for 18 hours under shaking conditions. The culture was centrifuged to obtain the proliferated transformants which were then treated with a conventional alkali-SDS method to isolate a recombinant DNA. A portion of the recombinant DNA isolated was analyzed by dideoxyribonucleotide sequencing, revealing that the recombinant DNA contained restriction sites of *Eco* RI and *Hind* III at the 5'- and 3'-termini of SEQ ID NO: 18, respectively, and a DNA containing a methionine codon for initiating polypeptide synthesis and a TAG codon for terminating polypeptide synthesis, which were located in just before and after the N- and C-termini of the amino acid sequence as shown in parallel in SEQ ID NO: 18. The recombinant DNA contained the nucleotide sequences of SEQ ID NOs: 1 to 5. These amino acid sequences were encoded by nucleotides 46-63, 85-102, 394-414, 148-162, and 211-225 in SEQ ID NO: 18.

The remaining portion of the recombinant DNA was in a conventional manner cleaved with restriction enzymes of *Eco* RI and *Hind* II, and the resulting 0.1  $\mu$ g of an *Eco* RI-*Hind* III DNA fragments, obtained by using "DNA LIGATION KIT VER 2", a DNA ligation kit commercialized by Takara Shuzo Co., Ltd., Tokyo, Japan, and 10 ng of pKK223-3, a plasmid vector commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been cleaved with a restriction enzyme were linked together, by incubating at 16°C for 30 min to obtain an autonomously-replicable recombinant DNA, pKGFMH2. Using competent cell method, an *Escherichia coli* Y1090 strain, ATCC 37197, was transformed using the recombinant DNA pKGFMH2, and the resulting transformant, KGFMH2, was inoculated to L-broth (pH 7.2) containing 50  $\mu$ g/ml ampicillin, and cultured at 37°C for 18 hours under shaking conditions. The culture was centrifuged to collect the proliferated transformants, followed by applying a conventional SDS-alkali method to a portion of the transformants to extract the recombinant DNA pKGFMH2. Dideoxyribonucleotide sequencing analysis revealed that, as shown in FIG. 5, KGFMH2 cDNA containing the nucleotide sequence of SEQ ID NO: 18 was linked to the downstream of the Tac promoter in the recombinant DNA pKGFMH2.

Ampicillin was added to L-broth (pH 7.2), which had been sterilized by autoclaving, to give a concentration of 50  $\mu$ g/ml, cooled to 37°C, and inoculated with the transformant KGFMH2, followed by the culture at 37°C for 18 hours. Eighteen liters of a fresh preparation of the same culture medium was placed in a 20-l jar fermenter, similarly sterilized as above, admixed with ampicillin, cooled to 37°C, and inoculated with one v/v % of the seed culture obtained in the above, followed by the culture at 37°C for 8 hours under aeration-agitation conditions. The resulting culture was centrifuged to collect the cultured cells which were then suspended in a mixture solution (pH 7.3) containing 150 mM sodium chloride, 16 mM disodium hydrogenphosphate, and 4 mM sodium dihydrogenphosphate, disrupted by ultrasonication, and centrifuged to remove cell disruptant, and this yielded an about two liters of a supernatant.

To an about two liters of the supernatant was added 10 mM phosphate buffer (pH 7.3) containing ammonium sulfate to give a 40% ammonium saturation. The resulting sediment was removed by centrifugation, and the supernatant

was mixed with ammonium sulfate to give an 85% ammonium saturation, allowed to stand at 4°C for 18 hours, and centrifuged at about 8,000 rpm for 30 min to obtain a newly formed sediment. The sediment thus obtained was dissolved in 10 mM phosphate buffer (pH 6.6) containing 1.5 M ammonium sulfate to give a total volume of about 1,300 ml, and the solution was filtered, and fed to a column packed with about 800 ml of "PHENYL SEPHAROSE CL-6B", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, followed by washing the column with a fresh preparation of the same buffer and feeding to the column a linear gradient buffer of ammonium sulfate decreasing from 1.5 M to 0 M in 10 mM phosphate buffer (pH 6.6) at an SV (space velocity) 1.5. Fractions eluted at around 1 M ammonium sulfate were pooled, concentrated using a membrane filter, and dialyzed against 10 mM phosphate buffer (pH 6.5) at 4°C for 18 hours. The dialyzed solution was fed to a column packed with about 55 ml of "DEAE-5PW", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been equilibrated with 10 mM phosphate buffer (pH 6.5). The column was washed with a fresh preparation of the same buffer, and fed with a linear gradient buffer of sodium chloride increasing from 0 M to 0.5 M in 10 mM phosphate buffer (pH 6.5) at SV 5.5, followed by collecting fractions eluted at around 0.2 M sodium chloride. Thereafter, the fractions were pooled and concentrated similarly as above up to give an about nine milliliters, followed by dialyzing the concentrate against PBS (phosphate buffered saline) at 4°C for 18 hours, and feeding the dialyzed solution to a column packed with "SUPERDEX 75", a gel commercialized by Pharmacia LKB Biotechnology AB, Uppsala, Sweden, which had been equilibrated with a fresh preparation of the same PBS. The column was fed with a fresh preparation of the same PBS to collect fractions with an IFN- $\gamma$  inducing activity, and the fractions were pooled and concentrated with a membrane filter to obtain a purified mouse IL-18 in a yield of about 350  $\mu$ g/l culture.

According to the method in Japanese Patent Kokai No. 27,189/96, the purified mouse IL-18 was analyzed and determined for biological activity and physicochemical property as indicated below: Culturing mouse spleen cells, collected by a conventional manner, under different concentrations of the mouse IL-18 resulted in an IFN- $\gamma$  production depending on the concentrations of the mouse IL-18, and this revealed that the mouse IL-18 has an activity of inducing IFN- $\gamma$  production by spleen cells as an immunocompetent cell. In accordance with the method as reported by U. K. Laemmli in *Nature*, Vol. 227, pp. 680-685 (1970), the purified human IL-18 was subjected to SDS-PAGE under non-reducing conditions, resulting in a major band with an IFN- $\gamma$  inducing activity at a position corresponding to 19,000 $\pm$ 5,000 daltons. The N-terminal region of the mouse IL-18 contained the amino acid sequence of SEQ ID NO: 19 which corresponded to the N-terminal region of SEQ ID NO: 18.

With reference to Experiment 7, the biological activity of the IL-18 according to the present invention will be described in more detail, and Experiment 8 describes the cytotoxicity of the IL-18:

#### Experiment 7

##### Biological activity

##### Experiment 7-1

##### Induction of GM-CSF production

Using a heparinized syringe, blood was collected from a healthy volunteer and diluted two fold with serum-free RPMI 1640 medium (pH 7.4). The diluent was overlaid on a ficoll and centrifuged, and the collected lymphocytes were washed with RPMI 1640 medium (pH 7.4) supplemented with 10 v/v % fetal calf serum, and suspended in a fresh preparation of the same medium to give a cell density of  $1 \times 10^6$  cells/ml, followed by distributing the cell suspension to a 12-well microplate by two ml/well.

Using RPMI 1640 medium (pH 7.4) supplemented with 10 v/v % fetal calf serum, an IL-18 preparation obtained by the method in Experiment 1 was prepared into a one  $\mu$ g/ml solution which was then distributed to the above microplate by 20-200  $\mu$ l/well. To the microplate was further added a fresh preparation of the same buffer, supplemented with 500  $\mu$ l/ml of Concanavalin A, by 10  $\mu$ l/well, followed by the incubation at 37°C for 48 hours in a 5 v/v % CO<sub>2</sub> incubator. After completion of the culture, supernatants in each well were sampled by 0.1 ml/well, and determined for GM-CSF content using a conventional enzyme immunoassay. In parallel, a culture system free of IL-18 as a control was provided and treated similarly as above. The data is in Table 1:

Table 1

IL-18* (nM)	GM-CSF yield (pg/ml)
0	510
0.7	2.150

Table 1 (continued)

IL-18* (nM)	GM-CSF yield (pg/ml)
2.8	3,050
5.6	3,950
Note: The symbol "*" means that IL-18 was added to the culture system in the presence of 2.5 µg/ml of Concanavalin A.	

The results in Table 1 indicate that lymphocytes as an immunocompetent cell produced GM-CSF depending on the concentration of IL-18 when contacted with IL-18 in the presence of Concanavalin A as a cofactor. It was also confirmed that all of the IL-18 preparations and functional equivalents thereof, which were obtained by the methods in Experiments 2 to 5, induced GM-CSF production even when used alone similarly as above. An IL-18 preparation obtained by the method in Experiment 6 was tested in accordance with Experiment 7-1 except that the human lymphocytes used in the experiment were replaced with spleen cells prepared from mouse by a conventional manner, revealing that the IL-18 preparation also induced GM-CSF production.

#### Experiment 7-2

#### Inhibition of osteoclast formation

#### Experiment 7-2(a)

As reported by T. J. Martin and K. W. Ng in *Journal of Cellular Biochemistry*, Vol. 56, pp. 357-366 (1994), it is considered requisite for contacting osteoclastic precursor cells, derived from hematopoietic stem cells, with osteoblasts or bone marrow stromas to generally differentiate osteoclastic precursor cells into mature osteoclasts. As described by G. D. Roodman in *Endocrine Reviews*, Vol. 17, No. 4, pp. 308-332 (1996), it is generally recognized that osteoclasts have characters of multinucleated cells, tartaric acid-resistant acid phosphatase (hereinafter abbreviated as "TRAP") activity, and a calcitonin receptor. In a co-culture system of osteoblasts and bone marrow cells as reported by Nobuyuki UDAGAWA et al., in *Journal of Experimental Medicine*, Vol. 182, pp. 1,461-1,468 (1995), these cells respond to factors such as  $1\alpha,25$ -dihydroxyvitamin D<sub>3</sub>, prostaglandin E<sub>2</sub>, adrenocortical hormone, interleukin 1, interleukin 6, and interleukin 11, to form osteoclast-like cells (hereinafter may be abbreviated as "OCL"). The formed OCL has characters of osteoclasts *in vivo*. Therefore, the co-culture system well reflects *in vitro* the processes of osteoclast formation *in vivo*. Using this system, experiments for osteoclast formation and osteoclastogenic inhibitory agents can be carried out.

The osteoclastogenic inhibitory activity of the IL-18 according to the present invention was studied using the above co-culture system. The osteoblasts used in this experiment were prepared in a conventional manner by treating a newborn mouse calvaria with 0.1 w/v % collagenase commercialized by Worthington Biochemical Co., Freefold, Australia, and 0.2 w/v % dispase commercialized by Godo Shusei Co., Ltd., Tokyo, Japan. The bone marrow cells were prepared from a mature mouse in a conventional manner. As a negative control,  $2 \times 10^4$  cells of a primary cell culture of osteoblasts and  $5 \times 10^5$  cells of bone marrow cells were co-cultured in each well of a 48-well microplate containing 0.4 ml/well of  $\alpha$ -MEM medium supplemented with 10 v/v % fetal calf serum (hereinafter designated as "Medium" throughout Experiment 4-2) at 37°C for seven days in a 5 v/v % CO<sub>2</sub> incubator. As a positive control, the above two-types of cells were co-cultured similarly as in the negative control except that they were cultured in other wells containing  $10^{-8}$ M of  $1\alpha,25$ -dihydroxyvitamin D<sub>3</sub> commercialized by Wako Pure Chemicals, Tokyo, Japan, and  $10^{-7}$ M of prostaglandin E<sub>2</sub> commercialized by Sigma Chemical Company, Missouri, USA. The aforesaid two-types of cells were co-cultured similarly as in the positive control except that they were cultured in other wells containing  $1\alpha,25$ -dihydroxyvitamin D<sub>3</sub> commercialized by Wako Pure Chemicals, Tokyo, Japan, and prostaglandin E<sub>2</sub> commercialized by Sigma Chemical Company, Missouri, USA., in the same concentrations as used in the positive control, and a concentration of 0.01-10 ng/ml of an IL-18 preparation prepared by the method in Experiment 6. In every co-culture system, the media in each well were replaced with fresh preparations of the same media used in the co-culture systems on the 3rd day after the initiation of each culture. According to the method by Nobuyuki UDAGAWA in *Journal of Experimental Medicine*, Vol. 182, pp. 1,461-1,468 (1995), the cells on the 6th day after the initiation of each culture were fixed and stained based on TRAP activity, followed by counting the stained cells (hereinafter called "TRAP-positive cells") per well. Throughout Experiment 4-2, quadruplet wells under the same conditions were provided for each co-culture system, and the mean value for the TRAP-positive cells per well in each system was calculated. The results are in Table 2:

Table 2

IL-18 (ng/ml)	Osteoclastgenic formation factor*1	Number of TRAP-positive cells per well*2
0	-	2
0	+	110
0.01	+	114
0.1	+	111
0.5	+	106
1	+	63
2	+	29
4	+	12
8	+	2
10	+	2

Note: \*1: The symbols of "+" and "-" show co-culture systems with and without 10<sup>-8</sup>M 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub> and 10<sup>-7</sup>M prostaglandin E<sub>2</sub>, respectively.  
\*2: It shows a mean value of the data from quadruplet wells cultured under the same conditions.

As shown in Table 2, the formation of TRAP-positive cells was not substantially observed in the negative control, but the distinct formation was observed in the positive control. In the co-culture systems, i.e., the positive control supplemented additionally with IL-18, the formation of TRAP-positive cells was inhibited depending on the concentration of IL-18, and the maximum inhibition, i.e., a level equal to that in the negative control, was found at eight ng/ml or more of IL-18. These data strongly indicates that IL-18 has a concrete activity of inhibiting OCL formation *in vitro* and also inhibits osteoclast formation.

#### Experiment 7-2(b)

As described hereinbefore, it was confirmed that there exist factors that induce the formation of osteoclast-like cells in the co-culture systems used throughout Experiment 7-2. Therefore, in this Experiment 7-2(b), it was studied whether the inhibitory activity of IL-18 on osteoclast formation observed in Experiment 7-2(a) was specific to some factors or not; the osteoclast-like cells were cultured by the same method as used in the negative control in Experiment 7-2(a) except for using a medium supplemented with  $10^{-8}$ M  $1\alpha,25$ -dihydroxyvitamin  $D_3$ ,  $10^{-7}$ M prostaglandin  $E_2$ , 200 ng/ml parathyroid hormone, 100 ng/ml interleukin 1, or 20 ng/ml interleukin 11. These culture systems were for positive controls. In parallel, the cells were cultured in other wells by the same method used in the positive controls except for using a medium containing 10 ng/ml of an IL-18 preparation obtained by the method in Experiment 6, in addition to any one of the above factors at the same concentration. After completion of the cultures, TRAP-positive cells in each well were counted, and the numbers were compared similarly as in Experiment 7-2(a). The results are in Table 3:

Table 3

Osteoclast formation factor*1 (concentration)	IL-18*2	Number of TRAP-positive cells per well*3
D <sub>3</sub> (10 <sup>-8</sup> M)	-	94
	+	3
PGE <sub>2</sub> (10 <sup>-7</sup> M)	-	77
	+	3
PTH (200 ng/ml)	-	63
	+	3
IL-11 (100 ng/ml)	-	84
	+	3
IL-1 (20 ng/ml)	-	71
	+	3

Note: \*1: D<sub>3</sub>, PGE<sub>2</sub>, PTH, IL-11, and IL-1 are respectively 1 $\alpha$ ,25-dihydroxyvitamin D<sub>3</sub>, prostaglandin E<sub>2</sub>, parathyroid hormone, interleukin-11, and interleukin-1 which were added to wells to give the concentrations as indicated in parentheses.

\*2: The symbol "+" means that IL-18 was added to a well to give a concentration of 10 ng/ml, and the symbol "-" means that IL-18 was not added to.

\*3: It shows a mean value of the data from quadruplet wells cultured under the same conditions.

As shown in Table 3, a distinct formation of TRAP-positive cells was observed in every positive control, but the formation was almost completely inhibited in the presence of IL-18. This strongly indicates that IL-18 has a wide and general activity of inhibiting osteoclast formation independently of osteoclast-formation-related factors.

5 Experiment 7-2(c)

It was studied whether the osteoclastgenic inhibition by IL-18, confirmed in Experiments 7-2(a) and 7-2(b), was caused by the action of the IL-18-induced GM-CSF. For positive and negative controls, the same co-culture systems employed in Experiment 7-2(a) were used. Using other wells, the co-culture of osteoblasts and bone marrow cells was  
10 carried out similarly as the method used for the positive controls except for using a medium supplemented with  $1\alpha$ , 25-dihydroxyvitamin D<sub>3</sub> and prostaglandin E<sub>2</sub> at the same concentrations used in the positive control, and with (i) 10  $\mu$ g/ml of an anti-mouse GM-CSF polyclonal antibody commercialized by R&D Systems, Minnesota, USA, (ii) 10 ng/ml of an IL-18 preparation obtained by the method in Experiment 6, (iii) (ii) plus 10  $\mu$ g/ml of an anti-mouse polyclonal antibody, (iv) 0.1 ng/ml of a mouse GM-CSF commercialized by R&D Systems, Minnesota, USA, or (v) (iv) plus 10  $\mu$ g/  
15 ml of an anti-mouse GM-CSF polyclonal antibody. After completion of the culture, TRAP-positive cells in each well were counted, and the numbers were compared similarly as in Experiment 7-2(a). The data is shown in Table 4 where the symbols "i" to "v" coincide with those used in the co-culture systems other than the control systems.

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Table 4

Culture system <sup>1</sup>	Osteoclastogenic factor <sup>2</sup>	IL-18 <sup>3</sup>	GM-CSF <sup>4</sup>	Anti-GM-CSF antibody <sup>5</sup>	Number of TRAP-positive cells per well <sup>6</sup>
N	-	-	-	-	3
P	+	-	-	-	122
i	+	-	-	+	112
11	+	+	-	-	3
111	+	+	-	+	111
iv	+	-	+	-	4
v	+	-	+	+	106

Note: <sup>1</sup>1; where the symbols "N" and "P" mean negative and positive controls, respectively, and the symbols "i" to "v" correspond to those in the five types co-culture systems used.

<sup>2</sup>2; where the symbol "+" means that  $1\alpha,25$ -dihydroxyvitamin D<sub>3</sub> and prostaglandin E<sub>2</sub> were respectively added to a well to give respective concentrations of  $10^{-8}$ M and  $10^{-7}$ M, and the symbol "-" means that these compounds were not added to.

<sup>3</sup>3; The symbol "+" means that IL-18 was added to a well to give a concentration of 10 ng/ml, and the symbol "-" means that IL-18 was not added to.

<sup>4</sup>4; The symbol "+" means that GM-CSF was added to a well to give a concentration of 0.1 ng/ml, and the symbol "-" means that GM-CSF was not added to.

<sup>5</sup>5; The symbol "+" means that an anti-GM-CSF polyclonal antibody was added to a well to give a concentration of 10  $\mu$ g/ml, and the symbol "-" means that the polyclonal antibody was not added to.

As shown in Table 4, the formation of TRAP-positive cells was almost completely inhibited by IL-18, cf., the co-culture system (ii), but the inhibition was almost completely inhibited by the addition of the anti-mouse polyclonal antibody, cf., the co-culture system (iii). Mouse GM-CSF exhibited an activity of inhibiting the formation of TRAP-positive cells similar to IL-18, cf., the co-culture system (iv), and the inhibition was almost completely inhibited by the addition of the anti-mouse GM-CSF polyclonal antibody, cf., the co-culture system (v). The sole use of the anti-mouse GM-CSF polyclonal antibody gave no influence on the formation of TRAP-positive cells, cf., the co-culture system (i). These data strongly indicates that the osteoclastgenic inhibition by IL-18 was due to the action of the IL-18-induced GM-CSF.

## Experiment 8

### Acute toxicity test

Eight-week-old mice were in a conventional manner injected percutaneously, orally, or intraperitoneally with either of IL-18 preparations obtained by the methods in Experiments 1 to 6. The results showed that these IL-18 preparations had an LD<sub>50</sub> of about one mg/kg or more in mice independent of the route of administration. The data evidences that IL-18 can be incorporated into pharmaceuticals for warm-blooded animals in general and including humans without causing no serious side effects.

As described in *Nikkei Biotechnology Annual Report 1996*, pp. 498-499 (1995), published by Nikkei BP Publisher, Tokyo, Japan (1995), the IL-18-induced GM-CSF has not yet been clinically used in Japan, but applied clinically in USA and Europe. The fact would show that IL-18 has substantially no serious side effects. These facts indicate that the osteoclastgenic inhibitory agent according to the present invention can be successively administered to warm-blooded animals in general and including humans to induce osteoclast formation and exert a satisfactory therapeutic and/or prophylactic effect on osteoclast-related diseases without causing serious side effects.

The following Examples describe the present osteoclastgenic inhibitory agent according to the present invention:

### Example 1

#### Liquid

Either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in physiological saline containing one w/v % human serum albumin as a stabilizer to give a concentration of two mg/ml of the IL-18 preparation. The resulting solutions were in a conventional manner membrane filtered for sterilization into liquids.

The liquids have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of an injection, ophthalmic solution, or collunarium for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

### Example 2

#### Dry agent

Fifty milligrams of either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in 100 ml of physiological saline containing one w/v % purified gelatin as a stabilizer. The solutions thus obtained were in a conventional manner membrane filtered for sterilization, distributed to vials by one milliliter, lyophilized, and sealed with caps.

The products have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of a dry injection for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

### Example 3

#### Dry agent

Fifty milligrams of either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, was dissolved in 100 ml of physiological saline containing one w/v % trehalose as a stabilizer. The solutions were in a conventional manner membrane filtered for sterilization, distributed to vials by one milliliter, lyophilized, and sealed with caps.

The products have a satisfactory stability and can be arbitrarily used as ingredients for cell culture and agents in the form of a dry injection for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 4Ointment

5        **"HIVIS WAKO GEL® 104"**, a carboxyvinylpolymer commercialized by Wako Pure Chemical Industries, Ltd., Tokyo, Japan, and a high-purity trehalose were dissolved in a sterilized distilled water to give respective concentrations of 1.4 w/w % and 2.0 w/w %, and the solution was mixed to homogeneity with either of IL-18 preparations obtained by the methods in Experiments 1 to 6, and adjusted to pH 7.2 to obtain a paste containing about one mg of an IL-18 preparation per g of the product.

10       Each product thus obtained has a satisfactory spreadability and stability and can be arbitrarily used as an agent in the form of an ointment for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

Example 5Tablet

15        **"FINETOSE®"**, an anhydrous crystalline  $\alpha$ -maltose powder commercialized by Hayashibara Biochemical Laboratories, Inc., Okayama, Japan, was mixed to homogeneity with either of IL-18 preparations, obtained by the methods in Experiments 1 to 6, and **"LUMIN"** or 1-1'-1"-triheptyl-11-chinoly(4)•4•4'-pentamethinchynocyanine-1,1"-dijodide. The mixtures were in a conventional manner tableted to obtain tablets, about 200 mg weight each, containing an about two milligrams of either of the IL-18 preparations and an about two milligrams of LUMIN per tablet.

20       The products have a satisfactory swallowability, stability, and cell-activating activity and can be arbitrarily used as agents in the form of a tablet for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

25       As described above, the osteoclastgenic inhibitory agent according to the present invention effectively inhibits osteoclast formation. Therefore, the agent can be arbitrarily used as an ingredient for cell culture and agents for regulating bone resorption and for osteoclast-related diseases, directed to treat and/or prevent hypercalcemia, osteoclastoma, osteoporosis, etc.

30       Thus the present invention with these useful activities and functions is a significant invention that would greatly contribute to this field.

35       While there has been described what is at present considered to be the preferred embodiments of the invention, it will be understood the various modifications may be made therein, and it is intended to cover in the appended claims all such modifications as fall within the true spirits and scope of the invention.

Annex to the description

## SEQUENCE LISTING

## (1) INFORMATION FOR SEQ ID NO: 1:

## (i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 6 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asn Asp Gln Val Leu Phe  
 1 5

## (2) INFORMATION FOR SEQ ID NO: 2:

## (i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 6 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Phe Glu Asp Met Thr Asp  
 1 5

## (3) INFORMATION FOR SEQ ID NO: 3:

## (i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 7 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Phe Lys Leu Ile Leu Lys Lys  
 1 5

## (4) INFORMATION FOR SEQ ID NO: 4:

## (i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 5 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 4:

5 Met Tyr Lys Asp Ser  
1 5

(5) INFORMATION FOR SEQ ID NO: 5:

10 (i)SEQUENCE CHARACTERISTICS:  
(A)LENGTH: 5 amino acids  
(B)TYPE: amino acid  
(D)TOPOLOGY: linear

15 (ii)MOLECULE TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 5:

20 Ser Thr Leu Ser Cys  
1 5

(6) INFORMATION FOR SEQ ID NO: 6:

25 (i)SEQUENCE CHARACTERISTICS:  
(A)LENGTH: 157 amino acids  
(B)TYPE: amino acid  
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

30 (xi)SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn  
1 5 10 15  
35 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp  
20 25 30  
Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile  
35 40 45  
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
50 55 60  
40 Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile  
65 70 75 80  
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
85 90 95  
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys  
100 105 110  
45 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu  
115 120 125  
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
130 135 140  
50 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
145 150 155

(7) INFORMATION FOR SEQ ID NO: 7:

55 (i)SEQUENCE CHARACTERISTICS:  
(A)LENGTH: 157 amino acids

(B)TYPE: amino acid  
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Asn	Phe	Gly	Arg	Leu	His	Cys	Thr	Thr	Ala	Val	Ile	Arg	Asn	Ile	Asn
1				5					10					15	
Asp	Gln	Val	Leu	Phe	Val	Asp	Lys	Arg	Gln	Pro	Val	Phe	Glu	Asp	Met
			20					25					30		
Thr	Asp	Ile	Asp	Gln	Ser	Ala	Ser	Glu	Pro	Gln	Thr	Arg	Leu	Ile	Ile
		35					40					45			
Tyr	Met	Tyr	Lys	Asp	Ser	Glu	Val	Arg	Gly	Leu	Ala	Val	Thr	Leu	Ser
	50					55					60				
Val	Lys	Asp	Ser	Lys	Met	Ser	Thr	Leu	Ser	Cys	Lys	Asn	Lys	Ile	Ile
65				70						75					80
Ser	Phe	Glu	Glu	Met	Asp	Pro	Pro	Glu	Asn	Ile	Asp	Asp	Ile	Gln	Ser
				85					90					95	
Asp	Leu	Ile	Phe	Phe	Gln	Lys	Arg	Val	Pro	Gly	His	Asn	Lys	Met	Glu
			100					105					110		
Phe	Glu	Ser	Ser	Leu	Tyr	Glu	Gly	His	Phe	Leu	Ala	Cys	Gln	Lys	Glu
		115				120						125			
Asp	Asp	Ala	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Lys	Asp	Glu	Asn	Gly	Asp
	130					135					140				
Lys	Ser	Val	Met	Phe	Thr	Leu	Thr	Asn	Leu	His	Gln	Ser			
145					150					155					

(8)INFORMATION FOR SEQ ID NO: 8:

(i)SEQUENCE CHARACTERISTICS:  
(A)LENGTH: 471 base pairs  
(B)TYPE: nucleic acid  
(C)STRANDEDNESS: double  
(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: cDNA

(vi)ORIGINAL SOURCE:  
(A)ORGANISM: human  
(G)CELL TYPE: liver

(ix)FEATURE:  
(A)NAME/KEY: mat peptide  
(B)LOCATION: 1..471  
(C)IDENTIFICATION METHOD: E

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
1				5					10					15		
GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
		20						25					30			
ATG	ACT	GAT	TCT	GAC	TGT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144

	Met	Thr	Asp	Ser	Asp	Cys	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
			35					40					45				
5	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
	Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
			50				55					60					
	TCT	GTG	AAG	TGT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	TGT	GAG	AAC	AAA	ATT	240
	Ser	Val	Lys	Cys	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile	
						70					75					80	
10	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
	Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
					85					90					95		
	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
	Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
				100				105						110			
15	ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
	Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	
			115					120					125				
	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
	Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
20			130				135					140					
	GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
	Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				
	145					150					155						

(9) INFORMATION FOR SEQ ID NO: 9:

(i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 11 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: N-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Met	Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser
1				5					10	

(10) INFORMATION FOR SEQ ID NO: 10:

(i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 10 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: C-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp
1				5				10	

## (11) INFORMATION FOR SEQ ID NO: 11:

(i)SEQUENCE CHARACTERISTICS:  
 (A)LENGTH: 13 amino acids  
 (B)TYPE: amino acid  
 (D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: N-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg  
 1 5 10

## (12) INFORMATION FOR SEQ ID NO: 12:

(i)SEQUENCE CHARACTERISTICS:  
 (A)LENGTH: 14 amino acids  
 (B)TYPE: amino acid  
 (D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg  
 1 5 10

## (13) INFORMATION FOR SEQ ID NO: 13:

(i)SEQUENCE CHARACTERISTICS:  
 (A)LENGTH: 17 amino acids  
 (B)TYPE: amino acid  
 (D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: internal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
 1 5 10 15

## (14) INFORMATION FOR SEQ ID NO: 14:

(i)SEQUENCE CHARACTERISTICS:  
 (A)LENGTH: 471 base pairs  
 (B)TYPE: nucleic acid  
 (C)STRANDEDNESS: double  
 (D)TOPOLOGY: linear

(ii)MOLECULE TYPE: cDNA

(ix)FEATURE:

(A)NAME/KEY: mat peptide

(B)LOCATION: 1..471

(C)IDENTIFICATION METHOD: S

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 14:

TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
1				5				10						15		
GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
			20					25						30		
ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
			35				40					45				
ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
			50				55					60				
TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile	
				70				75						80		
ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
			85					90						95		
AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
			100					105						110		
ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	
			115				120					125				
AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
			130				135					140				
GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				
145				150							155					

(15) INFORMATION FOR SEQ ID NO: 15:

(i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 10 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: N-terminal fragment

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser
1				5				10	

## (16) INFORMATION FOR SEQ ID NO: 16:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 471 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

(A) NAME/KEY: mat peptide

(B) LOCATION: 1..471

(C) IDENTIFICATION METHOD: S

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
1			5					10						15		
GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
		20					25						30			
ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
		35					40					45				
ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
	50				55			60								
TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile	
65				70			75						80			
ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
			85				90						95			
AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
		100					105					110				
ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TCT	GAA	384
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu	
		115					120					125				
AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
	130					135					140					
GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				
145				150						155						

## (17) INFORMATION FOR SEQ ID NO: 17:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 11464 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii)MOLECULE TYPE: genomic DNA

(vi)ORIGINAL SOURCE:

(A)ORGANISM: human  
(G)CELL TYPE: placenta

(ix)FEATURE:

(A)NAME/KEY: 5' UTR  
(B)LOCATION: 1..3  
(C)IDENTIFICATION METHOD: E  
(A)NAME/KEY: leader peptide  
(B)LOCATION: 4..82  
(C)IDENTIFICATION METHOD: S  
(A)NAME/KEY: intron  
(B)LOCATION: 83..1453  
(C)IDENTIFICATION METHOD: E  
(A)NAME/KEY: leader peptide  
(B)LOCATION: 1454..1465  
(C)IDENTIFICATION METHOD: S  
(A)NAME/KEY: intron  
(B)LOCATION: 1466..4848  
(C)IDENTIFICATION METHOD: E  
(A)NAME/KEY: leader peptide  
(B)LOCATION: 4849..4865  
(C)IDENTIFICATION METHOD: S  
(A)NAME/KEY: mat peptide  
(B)LOCATION: 4866..4983  
(C)IDENTIFICATION METHOD: S  
(A)NAME/KEY: intron  
(B)LOCATION: 4984..6317  
(C)IDENTIFICATION METHOD: E  
(A)NAME/KEY: mat peptide  
(B)LOCATION: 6318..6451  
(C)IDENTIFICATION METHOD: S  
(A)NAME/KEY: intron  
(B)LOCATION: 6452..11224  
(C)IDENTIFICATION METHOD: E  
(A)NAME/KEY: mat peptide  
(B)LOCATION: 11225..11443  
(C)IDENTIFICATION METHOD: S  
(A)NAME/KEY: 3' UTR  
(B)LOCATION: 11444..11464  
(C)IDENTIFICATION METHOD: E

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 17:

AAG ATG GCT GCT GAA CCA GTA GAA GAC AAT TGC ATC AAC TTT GTG GCA	48
Met Ala Ala Glu Pro Val Glu Asp Asn Cys Ile Asn Phe Val Ala	
-35 -30 -25	
ATG AAA TTT ATT GAC AAT ACG CTT TAC TTT ATA G GTAAGG CTAATGCCAT	98
Met Lys Phe Ile Asp Asn Thr Leu Tyr Phe Ile Ala	
-20 -15 -10	
AGAACAAATA CCAGGTTTCAG ATAAATCTAT TCAATTAGAA AAGATGTTGT GAGGTGAACT	158
ATTAAGTGAC TCTTTGTGTC ACCAAATTTT ACTGTAATAT TAATGGCTCT TAAAAAATA	218
GTGGACCTCT AGAAATTAAC CACAACATGT CCAAGGTCTC AGCACCTTGT CACACCACGT	278
GTCCTGGCAC TTTAATCAGC AGTAGCTCAC TCTCCAGTTG GCAGTAAGTG CACATCATGA	338

	AAATCCCAGT	TTTCATGGGA	AAATCCCAGT	TTTCATTGGA	TTTCCATGGG	AAAAATCCCA	398
	GTACAAAAC	GGGTGCATTC	AGGAAATACA	ATTTCCCAA	GCAAATTGGC	AAATTATGTA	458
	AGAGATTCTC	TAAATTTAGA	GTTCCGTGAA	TTACACCATT	TTATGTAAAT	ATGTTTGACA	518
5	AGTAAAAATT	GATTCTTTTT	TTTTTTTTTCT	GTTGCCCAGG	CTGGAGTGCA	GTGGCACAAT	578
	CTCTGCTCAC	TGCAACCTCC	ACCTCCTGGG	TTCAAGCAAT	TCTCCTGCCT	CAGCCTTCTG	638
	AGTAGCTGGG	ACTACAGGTG	CATCCCGCCA	TGCCTGGCTA	ATTTTTTGGGT	ATTTTTTACTA	698
	GAGACAGGGT	TTTGGCATGT	TGTCCAGGCT	GGTCTTGAC	TCCTGATCTC	AGATGATCCT	758
	CCTGGCTCGG	GCTCCCAAAG	TGCTGGGATT	ACAGGCATGA	ACCACCACAC	ATGGCCTAAA	818
10	AATTGATTCT	TATGATTAAT	CTCCTGTGAA	CAATTTGGCT	TCATTTGAAA	GTTTGCCTTC	878
	ATTTGAAACC	TTCAATTTAAA	AGCCTGAGCA	ACAAAGTGAG	ACCCCATCTC	TACAAAAAAC	938
	TGCAAAATAT	CCTGTGGACA	CCTCCTACCT	TCTGTGGAGG	CTGAAGCAGG	AGGATCACTT	998
	GAGCCTAGGA	ATTTGAGCCT	GCAGTGAGCT	ATGATCCAC	CCCTACACTC	CAGCCTGCAT	1058
	GACAGTAGAC	CCTGACACAC	ACACACAAAA	AAAAACCTTC	ATAAAAAATT	ATTAGTTGAC	1118
	TTTTCTTAGG	TGACTTTCCG	TTTAAGCAAT	AAATTTAAAA	GTAAAATCTC	TAATTTTAGA	1178
15	AAATTTATTT	TTAGTTACAT	ATTGAAATTT	TTAAACCCTA	GGTTTAAGTT	TTATGTCTAA	1238
	ATTACCTGAG	AACACACTAA	GTCTGATAAG	CTTCATTTTA	TGGGCCTTTT	GGATGATTAT	1298
	ATAATATTCT	GATGAAAGCC	AAGACAGACC	CTTAAACCAT	AAAAATAGGA	GTTTCGAGAA	1358
	GAGGAGTAGC	AAAAGTAAAA	GCTAGAATGA	GATTGAATTC	TGAGTCGAAA	TACAAAATTT	1418
	TACATATTCT	GTTTCTCTCT	TTTTCCCCCT	CTTAG CT	GAA GAT GAT G	GTAAA	1470
				Ala Glu Asp Asp Glu			
20				-10			
	GTAGAAATGA	ATTTATTTTT	CTTTGCAAAC	TAAGTATCTG	CTTGAGACAC	ATCTATCTCA	1530
	CCATTGTCAG	CTGAGGAAAA	AAAAAAATGG	TTCTCATGCT	ACCAATCTGC	CTTCAAAGAA	1590
	ATGTGGACTC	AGTAGCACAG	CTTTGGAATG	AAGATGATCA	TAAGAGATAC	AAAGAAGAAC	1650
	CTCTAGCAAA	AGATGCTTCT	CTATGCCTTA	AAAAATTCTC	CAGCTCTTAG	AATCTACAAA	1710
25	ATAGACTTTG	CCTGTTTCAT	TGGTCCTAAG	ATTAGCATGA	AGCCATGGAT	TCTGTTGTAG	1770
	GGGGAGCGTT	GCATAGGAAA	AAGGGATTGA	AGCATTAGAA	TTGTCCAAAA	TCAGTAACAC	1830
	CTCCTCTCAG	AAATGCTTTG	GGAAGAAGCC	TGGAAGGTTT	CGGGTTGGTG	GTGGGGTGGG	1890
	GCAGAAAATT	CTGGAAGTAG	AGGAGATAGG	AATGGGTGGG	GCAAGAAGAC	CACATTCAGA	1950
	GGCCAAAAGC	TGAAAGAAAC	CATGGCATT	ATGATGAATT	CAGGGTAATT	CAGAATGGAA	2010
	GTAGAGTAGG	AGTAGGAGAC	TGGTGAGAGG	AGCTAGAGTG	ATAAACAGGG	TGTAGAGCAA	2070
30	GACGTTCTCT	CACCCCAAGA	TGTGAAATTT	GGACTTTATC	TTGGAGATAA	TAGGGTTAAT	2130
	TAAGCACAAAT	ATGTATTAGC	TAGGGTAAAG	ATTAGTTTGT	TGTAACAAAG	ACATCCAAG	2190
	ATACAGTAGC	TGAATAAGAT	AGAGAATTTT	TCTCTCAAAG	AAAGTCTAAG	TAGGCAGCTC	2250
	AGAAGTAGTA	TGGCTGGAAG	CAACCTGATG	ATATTGGGAC	CCCCAACCTT	CTTCAGTCTT	2310
	GTACCCATCA	TCCCTAGTT	GTTGATCTCA	CTCACATAGT	TGAAAATCAT	CATACTTCCT	2370
	GGGTTTCATAT	CCCAGTTATC	AAGAAAGGGT	CAAGAGAAGT	CAGGCTCATT	CCTTTCAAAG	2430
35	ACTCTAATTG	GAAGTTAAAC	ACATCAATCC	CCCTCATATT	CCATTGACTA	GAATTTAATC	2490
	ACATGGCCAC	ACCAAGTGCA	AGGAAATCTG	GAAAATATAA	TCTTTATTCC	AGGTAGCCAT	2550
	ATGACTCTTT	AAAATTCAGA	AATAATATAT	TTTTAAAATA	TCATTCTGGC	TTTGGTATAA	2610
	AGAATTGATG	GTGTGGGGTG	AGGAGGCCAA	AATTAAGGGT	TGAGAGCCTA	TTATTTTAGT	2670
	TATTACAAGA	AATGATGGTG	TCATGAATTA	AGGTAGACAT	AGGGGAGTGC	TGATGAGGAG	2730
40	CTGTGAATGG	ATTTTAGAAA	CACTTGAGAG	AATCAATAGG	ACATGATTTA	GGGTGAGATT	2790
	TGGAAAGGAG	AAGAAAGTAG	AAAAGATGAT	GCCTACATTT	TTCACTTAGG	CAATTTGTAC	2850
	CATTCAGTGA	AATAGGGAAC	ACAGGAGGAA	GAGCAGGTTT	TGGTGTATAC	AAAGAGGAGG	2910
	ATGGATGACG	CATTTTCGTTT	TGGATCTGAG	ATGTCTGTGG	AACGTCCTAG	TGGAGATGTC	2970
	CACAAACTCT	TCTACATGTG	GTTCTGAGTT	CAGGACACAG	ATTTGGGCTG	GAGATAGAGA	3030
	TATTGTAGGC	TTATACATAG	AAATGGCATT	TGAATCTATA	GAGATAAAAA	GACACATCAG	3090
45	AGGAAATGTG	TAAAGTGAGA	GAGGAAAAGC	CAAGTACTGT	GCTGGGGGGA	ATACCTACAT	3150
	TTAAAGGATG	CAGTAGAAAG	AAGCTAATAA	ACAACAGAGA	GCAGACTAAC	CAAAAGGGGA	3210
	GAAGAAAAAC	CAAGAGAATT	CCACCGACTC	CCAGGAGAGC	ATTTCAAGAT	TGAGGGGATA	3270
	GGTGTGTGT	TGAATTTTGC	AGCCTTGAGA	ATCAAGGGCC	AGAACACAGC	TTTTAGATTT	3330
	AGCAACAAGG	AGTTTGGTGA	TCTCAGTGAA	AGCAGCTTGA	TGGTGAAATG	GAGGCAGAGG	3390
50	CAGATTGCAA	TGAGTGAAAC	AGTGAATGGG	AAGTGAAGAA	ATGATACAGA	TAATTCTTGC	3450
	TAAAAGCTTG	GCTGTAAAAA	GGAGGAGAGA	AACAAGACTA	GCTGCAAAGT	GAGATTGGGT	3510
	TGATGGAGCA	GTTTTAAATC	TCAAAATAAA	GAGCTTTGTG	CTTTTTTGAT	TATGAAAATA	3570

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	ATGTGTTAAT	TGTAACATAAT	TGAGGCAATG	AAAAAAGATA	ATAATATGAA	AGATAAAAAT	3630
	ATAAAAACCA	CCCAGAAATA	ATGATAGCTA	CCATTTTGGT	ACAATATTTT	TACACTCCTT	3690
5	TCTATGTATA	TATACAGACA	CAGAAATGCT	TATATTTTTA	TTAAAAGGGA	TTGTACTATA	3750
	CCTAAGCTGC	TTTTTCTAGT	TAGTGATATA	TATGGACATC	TCTCCATGGC	AACGAGTAAT	3810
	TGCAGTTATA	TTAAGTTCAT	GATATTTTAC	AATAAGGGCA	TATCTTTGCC	CTTTTTATTT	3870
	AATCAATTCT	TAATTGGTGA	ATGTTTGT	CCAGTTTGT	GTTGTTATTA	ACAATGTTCC	3930
	CATAAGCATT	CCTGTACACC	AATGTTTACA	CATTTGTCTG	ATTTTTTCTT	CAGGATAAAA	3990
	CCCAGGAGGT	AGAATTGCTG	GGTTGATAGA	AGAGAAAGGA	TGATTGCCAA	ATTAAAGCTT	4050
10	CAGTAGAGGG	TACATGCCGA	GCACAAATGG	GATCAGCCCT	AGATACCAGA	AATGGCACTT	4110
	TCTCATTTCC	CCTTGGGACA	AAAGGGAGAG	AGGCAATAAC	TGTGCTGCCA	GAGTTAAATT	4170
	TGTACGTGGA	GAGCAGGAA	ATCATTTGCT	GAAAATGAAA	ACAGAGATGA	TGTTGTAGAG	4230
	GTCCTGAAGA	GAGCAAAGAA	AATTTGAAAT	TGCGGCTATC	AGCTATGGAA	GAGAGTGCTG	4290
	AACTGGAAAA	CAAAAGAAGT	ATTGACAATT	GGTATGCTTG	TAATGGCACC	GATTTGAACG	4350
	CTTGTGCCAT	TGTTCAACCAG	CAGCACTCAG	CAGCCAAGTT	TGGAGTTTTG	TAGCAGAAAG	4410
15	ACAAATAAGT	TAGGGATTTA	ATATCCTGGC	CAAATGGTAG	ACAAAATGAA	CTCTGAGATC	4470
	CAGCTGCACA	GGGAAGGAAG	GGAAGACGGG	AAGAGGTTAG	ATAGGAAATA	CAAGAGTCAG	4530
	GAGACTGGAA	GATGTTGTGA	TATTTAAGAA	CACATAGAGT	TGGAGTAAAA	GTGTAAGAAA	4590
	ACTAGAAGGG	TAAGAGACCG	GTCAGAAAGT	AGGCTATTTG	AAGTTAACAC	TTCAGAGGCA	4650
	GAGTAGTTCT	GAATGGTAAC	AAGAAATTGA	GTGTGCCTTT	GAGAGTAGGT	TAAAAAACAA	4710
20	TAGGCAACTT	TATTGTAGCT	ACTTCTGGAA	CAGAAGATTG	TCATTAATAG	TTTTAGAAAA	4770
	CTAAATATA	TAGCATACTT	ATTTGTCAAT	TAACAAAGAA	ACTATGTATT	TTTAAATGAG	4830
	ATTTAATGTT	TATTGTAG	AA AAC CTG GAA TCA GAT	TAC TTT GGC AAG CTT			4880
			Glu Asn Leu Glu Ser Asp	Tyr Phe Gly Lys Leu			
			-5	1	5		
25	GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT	GAC CAA GTT CTC TTC					4928
	Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn	Asp Gln Val Leu Phe					
			10	15	20		
	ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT	ATG ACT GAT TCT GAC					4976
	Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp	Met Thr Asp Ser Asp					
			25	30	35		
30	TGT AGA G GTATTTTTT TTAATTCGCA AACATAGAAA	TGACTAGCTA CTTCTTCCCA					5032
	Cys Arg Asp						
			40				
	TTCTGTTTTA	CTGCTTACAT	TGTTCCGTGC	TAGTCCCAAT	CCTCAGATGA	AAAGTCAACAG	5092
	GAGTGACAAT	AATTTCACTT	ACAGGAAACT	TTATAAGGCA	TCCACGTTTTT	TTAGTTGGGG	5152
	TAAAAAATTG	GATACAATAA	GACATTGCTA	GGGGTCATGC	CTCTCTGAGC	CTGCCTTTGA	5212
35	ATCACCAATC	CCTTTATTGT	GATTGCATTA	ACTGTTTAAA	ACCTCTATAG	TTGGATGCTT	5272
	AATCCCTGCT	TGTTACAGCT	GAAAATGCTG	ATAGTTTACC	AGGTGTGGTG	GCATCTATCT	5332
	GTAATCCTAG	CTACTTGGGA	GGCTCAAGCA	GGAGGATTGC	TTGAGGCCAG	GACTTTGAGG	5392
	CTGTAGTACA	CTGTGATCGT	ACCTGTGAAT	AGCCACTGCA	CTCCAGCCTG	GGTGATATAC	5452
	AGACCTTGTC	TCTAAAATTA	AAAAAAAAAA	AAAAAAAAAAC	CTTAGGAAAG	GAAATTGATC	5512
	AAGTCTACTG	TGCCTTCCAA	AACATGAATT	CCAAATATCA	AAGTTAGGCT	GAGTTGAAGC	5572
40	AGTGAATGTG	CATTCTTTAA	AAATACTGAA	TACTTACCTT	AACATATATT	TTAAATATTT	5632
	TATTTAGCAT	TTAAAAGTTA	AAAACAATCT	TTTAGAATTC	ATATCTTTAA	AATACTCAAA	5692
	AAAGTTGCAG	CGTGTGTGTT	GTAATACACA	TTAAACTGTG	GGGTTGTTTG	TTTGTTTGAG	5752
	ATGCAGTTTC	ACTCTGTCAC	CCAGGCTGAA	GTGCAGTGCA	GTGCAGTGGT	GTGATCTCGG	5812
	CTCACTACAA	CCTCCACCTC	CCACGTTCAA	GCGATTCTCA	TGCCTCAGTC	TCCCGAGTAG	5872
45	GTGGGATTAC	AGGCATGCAC	CACTTACACC	CGGCTAATTT	TTGTATTTTT	AGTAGAGCTG	5932
	GGGTTTCACC	ATGTTGGCCA	GGCTGGTCTC	AAACCCCTAA	CCTCAAGTGA	TCTGCCTGCC	5992
	TCAGCCTCCC	AAACAAACAA	ACAACCCAC	AGTTTAATAT	GTGTTACAAC	ACACATGCTG	6052
	CAACTTTTAT	GAGTATTTTA	ATGATATAGA	TTATAAAAGG	TTGTTTTTAA	CTTTTAAATG	6112
	CTGGGATTAC	AGGCATGAGC	CACTGTGCCA	GGCCTGAACT	GTGTTTTTAA	AAATGTCTGA	6172
	CCAGCTGTAC	ATAGTCTCCT	GCAGACTGGC	CAAGTCTCAA	AGTGGGAACA	GGTGTATTAA	6232
50	GGACTATCCT	TTGGTTAAAT	TTCCGCAAAT	GTTCTGTG	AAGAATTCTT	CTAACTAGAG	6292
	TTCTCATTTA	TTATATTTAT	TTCAG	AT AAT GCA CCC	CGG ACC ATA TTT ATT		6343
			Asp Asn Ala Pro	Arg Thr Ile Phe Ile			

55

							40					45					
	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	6391
	Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
5		50					55					60					
	TCT	GTG	AAG	TGT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	TGT	GAG	AAC	AAA	ATT	6439
	Ser	Val	Lys	Cys	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile	
	65					70					75				80		
	ATT	TCC	TTT	AAG	GTAAG	ACTGAGCCTT	ACTTTGTTTT	CAATCATGTT	AATATAATCA								6496
	Ile	Ser	Phe	Lys													
10	ATATAATTAG	AAATATAACA	TTATTTCTAA	TGTTAATATA	AGTAATGTAA	TTAGAAAACCT											6556
	CAAATATCCT	CAGACCAACC	TTTTGTCTAG	AACAGAAATA	ACAAGAAGCA	GAGAACCATT											6616
	AAAGTGAATA	CTTACTAAAA	ATTATCAAAC	TCTTTACCTA	TTGTGATAAT	GATGGTTTTT											6676
	CTGAGCCTGT	CACAGGGGAA	GAGGAGATAC	AACACTTGTT	TTATGACCTG	CATCTCCTGA											6736
	ACAATCAGTC	TTTATACAAA	TAATAATGTA	GAATACATAT	GTGAGTTATA	CATTTAAGAA											6796
15	TAACATGTGA	CTTTCCAGAA	TGAGTTCTGC	TATGAAGAAT	GAAGCTAATT	ATCCTTCTAT											6856
	ATTTCTACAC	CTTTGTAAAT	TATGATAATA	TTTTAATCCC	TAGTTGTTTT	GTTGCTGATC											6916
	CTTAGCCTAA	GTCTTAGACA	CAAGCTTCAG	CTTCCAGTTG	ATGTATGTTA	TTTTTAATGT											6976
	TAATCTAATT	GAATAAAAGT	TATGAGATCA	GCTGTAAAAG	TAATGCTATA	ATTATCTTCA											7036
	AGCCAGGTAT	AAAGTATTTT	TGGCCTCTAC	TTTTTCTCTA	TTATTCTCCA	TTATTATTCT											7096
20	CTATTATTTT	TCTCTATTTT	CTCCATTATT	GTTAGATAAA	CCACAATTAA	CTATAGCTAC											7156
	AGACTGAGCC	AGTAAGAGTA	GCCAGGGATG	CTTACAAATT	GGCAATGCTT	CAGAGGAGAA											7216
	TTCCATGTCA	TGAAGACTCT	TTTTGAGTGG	AGATTTGCCA	ATAAATATCC	GCTTTCATGC											7276
	CCACCCAGTC	CCCACTGAAA	GACAGTTAGG	ATATGACCTT	AGTGAAGGTA	CCAAGGGGCA											7336
	ACTTGGTAGG	GAGAAAAAAG	CCACTCTAAA	ATATAATCCA	AGTAAGAACA	GTGCATATGC											7396
	AACAGATACA	GCCCCCAGAC	AAATCCCTCA	GCTATCTCCC	TCCAACCAGA	GTGCCACCCC											7456
25	TTCAGGTGAC	AATTTGGAGT	CCCCATTCTA	GACCTGACAG	GCAGCTTAGT	TATCAAAATA											7516
	GCATAAGAGG	CCTGGGATGG	AAGGGTAGGG	TGGAAAGGGT	TAAGCATGCT	GTTACTGAAC											7576
	AACATAATTA	GAAGGGAAGG	AGATGGCCAA	GCTCAAGCTA	TGTGGGATAG	AGGAAAACCTC											7636
	AGCTGCAGAG	GCAGATTCAG	AAACTGGGAT	AAGTCCGAAC	CTACAGGTGG	ATTCTTGTTG											7696
	AGGGAGACTG	GTGAAAATGT	TAAGAAGATG	GAAATAATGC	TTGGCACTTA	GTAGGAACCTG											7756
	GGCAAATCCA	TATTTGGGGG	AGCCTGAAGT	TTATTCAATT	TTGATGGCCC	TTTTAAATAA											7816
30	AAAGAATGTG	GCTGGGCGTG	GTGGCTCACA	CCTGTAATCC	CAGCACTTTG	GGAGGCCGAG											7876
	GGGGGCGGAT	CACCTGAAGT	CAGGAGTTCA	AGACCAGCCT	GACCAACATG	GAGAAACCCC											7936
	ATCTCTACTA	AAAATACAAA	ATTAGCTGGG	CGTGGTGGCA	TATGCCTGTA	ATCCCAGCTA											7996
	CTCGGGAGGC	TGAGGCAGGA	GAATCTTTTG	AACCCGGGAG	GCAGAGGTTG	CGATGAGCCT											8056
	AGATCGTGCC	ATTGCACTCC	AGCCTGGGCA	ACAAGAGCAA	AACTCGGTCT	CAAAAAAAAAA											8116
35	AAAAAAAAAAG	TGAAATTAAC	CAAAGGCATT	AGCTTAATAA	TTTAATACTG	TTTTTAAGTA											8176
	GGGCGGGGGG	TGGCTGGAAG	AGATCTGTGT	AAATGAGGGA	ATCTGACATT	TAAGCTTCAT											8236
	CAGCATCATA	GCAAATCTGC	TTCTGGAAGG	AACTCAATAA	ATATTAGTTG	GAGGGGGGGA											8296
	GAGAGTGAGG	GGTGGACTAG	GACCAGTTTT	AGCCCTTGTC	TTTAATCCCT	TTTCCTGCCA											8356
	CTAATAAGGA	TCTTAGCAGT	GGTTATAAAA	GTGGCCTAGG	TTCTAGATAA	TAAGATACAA											8416
	CAGGCCAGGC	ACAGTGGCTC	ATGCCTATAA	TCCCAGCACT	TTGGGAGGGC	AAGGCGAGTG											8476
40	TCTCACTTGA	GATCAGGAGT	TCAAGACCAG	CCTGGCCAGC	ATGGCGATAC	TCTGTCTCTA											8536
	CTAAAAAAAAA	TACAAAAATT	AGCCAGGCAT	GGTGGCATGC	ACCTGTAATC	CCAGCTACTC											8596
	GTGAGCCTGA	GGCAGAAGAA	TCGCTTGAAA	CCAGGAGGTG	TAGGCTGCAG	TGAGCTGAGA											8656
	TCGCACCACT	GCACTCCAGC	CTGGGCGACA	GAATGAGACT	TTGTCTCAAA	AAAAGAAAAA											8716
	GATACAACAG	GCTACCCTTA	TGTGCTCACC	TTTCACTGTT	GATTACTAGC	TATAAAGTCC											8776
45	TATAAAGTTC	TTTGGTCAAG	AACCTTGACA	ACACTAAGAG	GGATTTGCTT	TGAGAGGTTA											8836
	CTGTCAGAGT	CTGTTTCATA	TATATACATA	TACATGTATA	TATGTATCTA	TATCCAGGCT											8896
	TGGCCAGGGT	TCCCTCAGAC	TTTCCAGTGC	ACTTGGGAGA	TGTTAGGTCA	ATATCAACTT											8956
	TCCCTGGATT	CAGATTCAAC	CCCTTCTGAT	GTAAAAAATA	AAAAAAAAAA	GAAAGAAATC											9016
	CCTTTCCCTT	TGGAGCACTC	AAGTTTCACC	AGGTGGGGCT	TTCCAAGTTG	GGGGTTCTCC											9076
	AAGGTCATTG	GGATTGCTTT	CACATCCATT	TGCTATGTAC	CTTCCCTATG	ATGGCTGGGA											9136
50	GTGGTCAACA	TCAAAACTAG	GAAAGCTACT	GCCCAAGGAT	GTCCTTACCT	CTATTCTGAA											9196
	ATGTGCAATA	AGTGTGATTA	AAGAGATTGC	CTGTTCTACC	TATCCACACT	CTCGCTTTCA											9256
	ACTGTAACCT	TCTTTTTTTC	TTTTTTTCTT	TTTTTCTTTT	TTTTTGAAAC	GGAGTCTCGC											9316

	TCTGTCGCCC	AGGCTAGAGT	GCAGTGGCAC	GATCTCAGCT	CACTGCAAGC	TCTGCCTCCC	9376
	GGGTTCACGC	CATTCTCCTG	CCTCACCCCTC	CCAAGCAGCT	GGGACTACAG	GCGCCTGCCA	9436
	CCATGCCCAG	CTAATTTTTT	GTATTTTTTAG	TAGAGACGGG	GTTTCACCGT	GTTAGCCAGG	9496
5	ATGGTCTCGA	TCTCCTGAAC	TTGTGATCCG	CCCGCCTCAG	CCTCCCAAAG	TGCTGGGATT	9556
	ACAGGCGTGA	GCCATCGCAC	CCGGCTCAAC	TGTAACCTTC	TATACTGGTT	CATCTTCCCC	9616
	TGTAATGTTA	CTAGAGCTTT	TGAAGTTTTG	GCTATGGATT	ATTTCTCATT	TATACATTAG	9676
	ATTTTCAGATT	AGTTCCAAAT	TGATGCCAC	AGCTTAGGGT	CTCTTCCTAA	ATTGTATATT	9736
	GTAGACAGCT	GCAGAAGTGG	GTGCCAATAG	GGGAAGTAGT	TTATACTTTC	ATCAACTTAG	9796
10	GACCCACACT	TGTTGATAAA	GAACAAAGGT	CAAGAGTTAT	GACTACTGAT	TCCACAACCTG	9856
	ATTGAGAAGT	TGGAGATAAC	CCCGTGACCT	CTGCCATCCA	GAGTCTTTCA	GGCATCTTTG	9916
	AAGGATGAAG	AAATGCTATT	TTAATTTTTG	AGGTTTCTCT	ATCAGTGCTT	AGGATCATGG	9976
	GAATCTGTGC	TGCCATGAGG	CCAAAATTAA	GTCCAAAACA	TCTACTGGTT	CCAGGATTAA	10036
	CATGGAAGAA	CCTTAGGTGG	TGCCACATG	TTCTGATCCA	TCCTGCAAAA	TAGACATGCT	10096
	GCACTAACAG	GAAAAGTGCA	GGCAGCACTA	CCAGTTGGAT	AACCTGCAAG	ATTATAGTTT	10156
15	CAAGTAATCT	AACCATTTCT	CACAAGGCCC	TATTCTGTGA	CTGAAACATA	CAAGAATCTG	10216
	CATTTGGCCT	TCTAAGGCAG	GGCCCAGCCA	AGGAGACCAT	ATTCAGGACA	GAAATTCAAG	10276
	ACTACTATGG	AACTGGAGTG	CTTGGCAGGG	AAGACAGAGT	CAAGGACTGC	CAACTGAGCC	10336
	AATACAGCAG	GCTTACACAG	GAACCCAGGG	CCTAGCCCTA	CAACAATTAT	TGGGTCTATT	10396
	CACTGTAAGT	TTTAATTTCA	GGCTCCACTG	AAAGAGTAAG	CTAAGATTCC	TGGCACTTTC	10456
20	TGTCTCTCTC	ACAGTTGGCT	CAGAAATGAG	AACTGGTCAG	GCCAGGCATG	GTGGCTTACA	10516
	CCTGGAATCC	CAGCACTTTG	GGAGGCCGAA	GTGGGAGGGT	CACTTGAGGC	CAGGAGTTCA	10576
	GGACCAGCTT	AGGCAACAAA	GTGAGATACC	CCCTGACCCC	TTCTCTACAA	AAATAAATTT	10636
	TAAAAATTAG	CCAAATGTGG	TGGTGTATAC	TTACAGTCCC	AGCTACTCAG	GAGGCTGAGG	10696
	CAGGGGGATT	GCTTGAGCCC	AGGAATTCAA	GGCTGCAGTG	AGCTATGATT	TCACCACTGC	10756
	ACTTCTGGCT	GGGCAACAGA	GCGAGACCCT	GTCTCAAAGC	AAAAAGAAAA	AGAAACTAGA	10816
25	ACTAGCCTAA	GTTTGTGGGA	GGAGGTCATC	ATCGTCTTTA	GCCGTGAATG	GTTATTATAG	10876
	AGGACAGAAA	TTGACATTAG	CCCAAAAAGC	TTGTGGTCTT	TGCTGGAAC	CTACTTAATC	10936
	TTGAGCAAAT	GTGGACACCA	CTCAATGGGA	GAGGAGAGAA	GTAAGCTGTT	TGATGTATAG	10996
	GGGAAAAC	GAGGCCTGGA	ACTGAATATG	CATCCCATGA	CAGGGAGAAT	AGGAGATTCTG	11056
	GAGTTAAGAA	GGAGAGGAGG	TCAGTACTGC	TGTTTCAGAGA	TTTTTTTTTAT	GTAACCTCTTG	11116
	AGAAGCAAAA	CTACTTTTGT	TCTGTTTGGT	AATATACTTC	AAAACAAACT	TCATATATTC	11176
30	AAATTGTTCA	TGTCCTGAAA	TAATTAGGTA	ATGTTTTTTT	CTCTATAG	GAA ATG AAT	11233
						Glu Met Asn	
						85	
	CCT CCT GAT AAC ATC AAG GAT ACA AAA AGT GAC ATC ATA TTC TTT CAG						11281
	Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp Ile Ile Phe Phe Glu						
35		90		95		100	
	AGA AGT GTC CCA GGA CAT GAT AAT AAG ATG CAA TTT GAA TCT TCA TCA						11329
	Arg Ser Val Pro Gly His Asp Asn Lys Met Gln Phe Glu Ser Ser Ser						
		105		110		115	
	TAC GAA GGA TAC TTT CTA GCT TGT GAA AAA GAG AGA GAC CTT TTT AAA						11377
	Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu Arg Asp Leu Phe Lys						
40		120		125		130	135
	CTC ATT TTG AAA AAA GAG GAT GAA TTG GGG GAT AGA TCT ATA ATG TTC						11425
	Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe						
		140		145		150	
	ACT GTT CAA AAC GAA GAC TAGCTATTAA AATTTTCATGC C						11464
45	Thr Val Gln Asn Glu Asp						
		155					

(18) INFORMATION FOR SEQ ID NO: 18:

## (1) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 471 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: cDNA to mRNA

(vi)ORIGINAL SOURCE:

(A)ORGANISM: mouse

(G)CELL TYPE: liver

(ix)FEATURE:

(A)NAME/KEY: mat peptide

(B)LOCATION: 1..471

(C)IDENTIFICATION METHOD: S

(xi)SEQUENCE DESCRIPTION: SEQ ID NO: 18:

15	AAC	TTT	GGC	CGA	CTT	CAC	TGT	ACA	ACC	GCA	GTA	ATA	CGG	AAT	ATA	AAT	48
	Asn	Phe	Gly	Arg	Leu	His	Cys	Thr	Thr	Ala	Val	Ile	Arg	Asn	Ile	Asn	
	1				5					10					15		
	GAC	CAA	GTT	CTC	TTC	GTT	GAC	AAA	AGA	CAG	CCT	GTG	TTC	GAG	GAT	ATG	96
	Asp	Gln	Val	Leu	Phe	Val	Asp	Lys	Arg	Gln	Pro	Val	Phe	Glu	Asp	Met	
20				20					25					30			
	ACT	GAT	ATT	GAT	CAA	AGT	GCC	AGT	GAA	CCC	CAG	ACC	AGA	CTG	ATA	ATA	144
	Thr	Asp	Ile	Asp	Gln	Ser	Ala	Ser	Glu	Pro	Gln	Thr	Arg	Leu	Ile	Ile	
				35				40					45				
	TAC	ATG	TAC	AAA	GAC	AGT	GAA	GTA	AGA	GGA	CTG	GCT	GTG	ACC	CTC	TCT	192
25	Tyr	Met	Tyr	Lys	Asp	Ser	Glu	Val	Arg	Gly	Leu	Ala	Val	Thr	Leu	Ser	
		50					55					60					
	GTG	AAG	GAT	AGT	AAA	ATG	TCT	ACC	CTC	TCC	TGT	AAG	AAC	AAG	ATC	ATT	240
	Val	Lys	Asp	Ser	Lys	Met	Ser	Thr	Leu	Ser	Cys	Lys	Asn	Lys	Ile	Ile	
	65				70					75					80		
	TCC	TTT	GAG	GAA	ATG	GAT	CCA	CCT	GAA	AAT	ATT	GAT	GAT	ATA	CAA	AGT	288
30	Ser	Phe	Glu	Glu	Met	Asp	Pro	Pro	Glu	Asn	Ile	Asp	Asp	Ile	Gln	Ser	
					85					90					95		
	GAT	CTC	ATA	TTC	TTT	CAG	AAA	CGT	GTT	CCA	GGA	CAC	AAC	AAG	ATG	GAG	336
	Asp	Leu	Ile	Phe	Phe	Gln	Lys	Arg	Val	Pro	Gly	His	Asn	Lys	Met	Glu	
				100					105					110			
35	TTT	GAA	TCT	TCA	CTG	TAT	GAA	GGA	CAC	TTT	CTT	GCT	TGC	CAA	AAG	GAA	384
	Phe	Glu	Ser	Ser	Leu	Tyr	Glu	Gly	His	Phe	Leu	Ala	Cys	Gln	Lys	Glu	
			115				120					125					
	GAT	GAT	GCT	TTC	AAA	CTC	ATT	CTG	AAA	AAA	AAG	GAT	GAA	AAT	CGG	GAT	432
	Asp	Asp	Ala	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Lys	Asp	Glu	Asn	Gly	Asp	
			130				135					140					
40	AAA	TCT	GTA	ATG	TTC	ACT	CTC	ACT	AAC	TTA	CAT	CAA	AGT				471
	Lys	Ser	Val	Met	Phe	Thr	Leu	Thr	Asn	Leu	His	Gln	Ser				
	145					150					155						

(19) INFORMATION FOR SEQ ID NO: 19:

(i)SEQUENCE CHARACTERISTICS:

(A)LENGTH: 9 amino acids

(B)TYPE: amino acid

(D)TOPOLOGY: linear

(ii)MOLECULE TYPE: peptide

(v)FRAGMENT TYPE: N-terminal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

Asn Phe Gly Arg Leu His Cys Thr Thr  
1 5

(20) INFORMATION FOR SEQ ID NO: 20:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 157 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn  
1 5 10 15  
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp  
20 25 30  
Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile  
35 40 45  
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
50 55 60  
Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile  
65 70 75 80  
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
85 90 95  
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys  
100 105 110  
Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu  
115 120 125  
Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
130 135 140  
Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
145 150 155

(21) INFORMATION FOR SEQ ID NO: 21:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 157 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn  
1 5 10 15  
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp  
20 25 30  
Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile  
35 40 45  
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
50 55 60

Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile  
 65 70 75 80  
 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
 85 90 95  
 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys  
 100 105 110  
 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu  
 115 120 125  
 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
 130 135 140  
 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 145 150 155

(22) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn  
 1 5 10 15  
 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp  
 20 25 30  
 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile  
 35 40 45  
 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
 50 55 60  
 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile  
 65 70 75 80  
 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
 85 90 95  
 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys  
 100 105 110  
 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu  
 115 120 125  
 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
 130 135 140  
 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 145 150 155

(23) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn  
 1 5 10 15  
 5 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp  
 20 25 30  
 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile  
 35 40 45  
 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
 50 55 60  
 10 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile  
 65 70 75 80  
 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
 85 90 95  
 15 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys  
 100 105 110  
 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu  
 115 120 125  
 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
 130 135 140  
 20 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 145 150 155

## (24) INFORMATION FOR SEQ ID NO: 24:

25 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: peptide

30 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn  
 1 5 10 15  
 35 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp  
 20 25 30  
 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile  
 35 40 45  
 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
 50 55 60  
 40 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ser Glu Asn Lys Ile  
 65 70 75 80  
 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
 85 90 95  
 45 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys  
 100 105 110  
 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu  
 115 120 125  
 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
 130 135 140  
 50 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 145 150 155

## (25) INFORMATION FOR SEQ ID NO: 25:

55 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids

(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
1				5					10					15	
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp
		20						25					30		
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile
		35					40					45			
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile
	50					55					60				
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile
65					70				75					80	
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
				85					90					95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100					105					110		
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu
		115					120					125			
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu
	130					135					140				
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp			
145					150					155					

(26) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 157 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
1				5					10					15	
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp
		20						25					30		
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile
		35					40					45			
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile
	50					55					60				
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile
65					70				75					80	
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
				85					90					95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100					105					110		
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu
		115					120					125			
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu

130                      135                      140  
 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 145                      150                      155

(27) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 157 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

1	Asn	Phe	Gly	Arg	Leu	His	Ala	Thr	Thr	Ala	Val	Ile	Arg	Asn	Ile	Asn
					5					10					15	
20	Asp	Gln	Val	Leu	Phe	Val	Asp	Lys	Arg	Gln	Pro	Val	Phe	Glu	Asp	Met
			20						25					30		
	Thr	Asp	Ile	Asp	Gln	Ser	Ala	Ser	Glu	Pro	Gln	Thr	Arg	Leu	Ile	Ile
			35					40					45			
	Tyr	Met	Tyr	Lys	Asp	Ser	Glu	Val	Arg	Gly	Leu	Ala	Val	Thr	Leu	Ser
		50					55					60				
25	Val	Lys	Asp	Ser	Lys	Met	Ser	Thr	Leu	Ser	Cys	Lys	Asn	Lys	Ile	Ile
	65					70					75					80
	Ser	Phe	Glu	Glu	Met	Asp	Pro	Pro	Glu	Asn	Ile	Asp	Asp	Ile	Gln	Ser
				85						90					95	
30	Asp	Leu	Ile	Phe	Phe	Gln	Lys	Arg	Val	Pro	Gly	His	Asn	Lys	Met	Glu
				100					105					110		
	Phe	Glu	Ser	Ser	Leu	Tyr	Glu	Gly	His	Phe	Leu	Ala	Cys	Gln	Lys	Glu
			115					120					125			
	Asp	Asp	Ala	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Lys	Asp	Glu	Asn	Gly	Asp
		130					135					140				
35	Lys	Ser	Val	Met	Phe	Thr	Leu	Thr	Asn	Leu	His	Gln	Ser			
	145					150					155					

(28) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 157 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

1	Asn	Phe	Gly	Arg	Leu	His	Cys	Thr	Thr	Ala	Val	Ile	Arg	Asn	Ile	Asn
					5					10					15	
50	Asp	Gln	Val	Leu	Phe	Val	Asp	Lys	Arg	Gln	Pro	Val	Phe	Glu	Asp	Met
			20						25					30		
	Thr	Asp	Ile	Asp	Gln	Ser	Ala	Ser	Glu	Pro	Gln	Thr	Arg	Leu	Ile	Ile
			35					40					45			
	Tyr	Met	Tyr	Lys	Asp	Ser	Glu	Val	Arg	Gly	Leu	Ala	Val	Thr	Leu	Ser
		50					55					60				
55	Val	Lys	Asp	Ser	Lys	Met	Ser	Thr	Leu	Ser	Cys	Lys	Asn	Lys	Ile	Ile

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	65				70				75				80			
	Ser	Phe	Glu	Glu	Met	Asp	Pro	Pro	Glu	Asn	Ile	Asp	Asp	Ile	Gln	Ser
5					85				90					95		
	Asp	Leu	Ile	Phe	Phe	Gln	Lys	Arg	Val	Pro	Gly	His	Asn	Lys	Met	Glu
				100					105					110		
	Phe	Glu	Ser	Ser	Leu	Tyr	Glu	Gly	His	Phe	Leu	Ala	Ser	Gln	Lys	Glu
			115					120					125			
10	Asp	Asp	Ala	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Lys	Asp	Glu	Asn	Gly	Asp
		130					135					140				
	Lys	Ser	Val	Met	Phe	Thr	Leu	Thr	Asn	Leu	His	Gln	Ser			
	145					150					155					

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## SEQUENCE LISTING

## (1) GENERAL INFORMATION:

## (i) APPLICANT:

NAME: KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU KAGAKU  
KENKYUJO

(ii) TITLE OF INVENTION: OSTEOCLASTGENIC INHIBITORY AGENT

(iii) NUMBER OF SEQUENCES: 28

## (iv) ADDRESS:

(A) ADDRESSEE: KABUSHIKI KAISHA HAYASHIBARA SEIBUTSU  
KAGAKU KENKYUJO

(B) STREET: 2-3, 1-CHOME, SHIMOISHII

(C) CITY: OKAYAMA

(E) COUNTRY: JAPAN

(F) POSTAL CODE (ZIP): 700

## (v) COMPUTER READABLE FORM:

(A) MEDIUM TYPE: Floppy disk

(B) COMPUTER: IBM PC compatible

(C) OPERATING SYSTEM: PC-DOS/MS-DOS

## (vii) PRIOR APPLICATION DATA:

(A1) APPLICATION NUMBER: JP 55,468/1997

(B1) FILING DATE: 25-FEB-1997

## (2) INFORMATION FOR SEQ ID NO: 1:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Asn Asp Gln Val Leu Phe

1

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## (3) INFORMATION FOR SEQ ID NO: 2:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 6 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Phe Glu Asp Met Thr Asp

1

5

## (4) INFORMATION FOR SEQ ID NO: 3:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 7 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

Phe Lys Leu Ile Leu Lys Lys  
1 5

(5) INFORMATION FOR SEQ ID NO: 4:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Met Tyr Lys Asp Ser  
1 5

(6) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 5 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Ser Thr Leu Ser Cys  
1 5

(7) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:  
(A) LENGTH: 157 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn  
1 5 10 15  
Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp  
20 25 30  
Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile  
35 40 45  
Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
50 55 60  
Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile  
65 70 75 80  
Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
85 90 95  
Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys

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Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu  
 115 120 125  
 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
 130 135 140  
 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 145 150 155

(8) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn  
 1 5 10 15  
 Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met  
 20 25 30  
 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile  
 35 40 45  
 Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser  
 50 55 60  
 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile  
 65 70 75 80  
 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser  
 85 90 95  
 Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu  
 100 105 110  
 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu  
 115 120 125  
 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp  
 130 135 140  
 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser  
 145 150 155

(9) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 471 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(vi) ORIGINAL SOURCE:  
 (A) ORGANISM: human  
 (G) CELL TYPE: liver

(ix) FEATURE:  
 (A) NAME/KEY: mat peptide  
 (B) LOCATION: 1..471  
 (C) IDENTIFICATION METHOD: E

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

TAC TTT GGC AAG CTT GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT

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	Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
	1				5					10					15		
	GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
5	Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
				20					25					30			
	ATG	ACT	GAT	TCT	GAC	TGT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
	Met	Thr	Asp	Ser	Asp	Cys	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
			35					40					45				
	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
10	Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
		50				55					60						
	TCT	GTG	AAG	TGT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	TGT	GAG	AAC	AAA	ATT	240
	Ser	Val	Lys	Cys	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile	
	65				70				75				80				
	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
15	Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
				85					90				95				
	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
	Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
				100				105					110				
	ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
20	Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	
			115					120				125					
	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
	Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
			130				135				140						
	GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
25	Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				
	145				150					155							

(10) INFORMATION FOR SEQ ID NO: 9:

30 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 11 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

35 (ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

40 Met Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser  
 1 5 10

(11) INFORMATION FOR SEQ ID NO: 10:

45 (i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 10 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

50 (v) FRAGMENT TYPE: C-terminal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

55 Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 1 5 10

(12) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 13 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg  
1 5 10

(13) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 14 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Thr Ile Phe Ile Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg  
1 5 10

(14) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 17 amino acids  
(B) TYPE: amino acid  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: internal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Ile Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
1 5 10 15

(15) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 471 base pairs  
(B) TYPE: nucleic acid  
(C) STRANDEDNESS: double  
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

(ix) FEATURE:

(A) NAME/KEY: mat peptide  
(B) LOCATION: 1..471  
(C) IDENTIFICATION METHOD: S

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

5	TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
	Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
	1				5					10					15		
	GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
	Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
			20					25					30				
10	ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
	Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
			35				40						45				
	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
	Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
			50				55					60					
15	TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
	Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile	
	65				70					75					80		
	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
	Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
				85				90					95				
20	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
	Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
				100				105					110				
	ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TGT	GAA	384
	Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	
			115				120					125					
25	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
	Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
			130				135					140					
	GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
	Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				
	145				150					155							

## (15) INFORMATION FOR SEQ ID NO: 15:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 10 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal fragment

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 15:

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser
1				5				10	

## (17) INFORMATION FOR SEQ ID NO: 16:

## (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 471 base pairs

(B) TYPE: nucleic acid

(C) STRANDEDNESS: double

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA

## (ix) FEATURE:

(A) NAME/KEY: mat peptide

(B) LOCATION: 1..471

## (C) IDENTIFICATION METHOD: S

## (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 16:

5	TAC	TTT	GGC	AAG	CTT	GAA	TCT	AAA	TTA	TCA	GTC	ATA	AGA	AAT	TTG	AAT	48
	Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	
	1			5					10						15		
	GAC	CAA	GTT	CTC	TTC	ATT	GAC	CAA	GGA	AAT	CGG	CCT	CTA	TTT	GAA	GAT	96
	Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	
10			20						25					30			
	ATG	ACT	GAT	TCT	GAC	TCT	AGA	GAT	AAT	GCA	CCC	CGG	ACC	ATA	TTT	ATT	144
	Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	
			35						40					45			
	ATA	AGT	ATG	TAT	AAA	GAT	AGC	CAG	CCT	AGA	GGT	ATG	GCT	GTA	ACT	ATC	192
	Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	
15			50						55				60				
	TCT	GTG	AAG	TCT	GAG	AAA	ATT	TCA	ACT	CTC	TCC	GCT	GAG	AAC	AAA	ATT	240
	Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ala	Glu	Asn	Lys	Ile	
	65					70						75				80	
	ATT	TCC	TTT	AAG	GAA	ATG	AAT	CCT	CCT	GAT	AAC	ATC	AAG	GAT	ACA	AAA	288
	Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	
20					85					90					95		
	AGT	GAC	ATC	ATA	TTC	TTT	CAG	AGA	AGT	GTC	CCA	GGA	CAT	GAT	AAT	AAG	336
	Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	
				100					105					110			
	ATG	CAA	TTT	GAA	TCT	TCA	TCA	TAC	GAA	GGA	TAC	TTT	CTA	GCT	TCT	GAA	384
25	Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu	
			115					120					125				
	AAA	GAG	AGA	GAC	CTT	TTT	AAA	CTC	ATT	TTG	AAA	AAA	GAG	GAT	GAA	TTG	432
	Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	
			130				135					140					
	GGG	GAT	AGA	TCT	ATA	ATG	TTC	ACT	GTT	CAA	AAC	GAA	GAC				471
30	Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				
	145					150					155						

## (18) INFORMATION FOR SEQ ID NO: 17:

35	(i) SEQUENCE CHARACTERISTICS:
	(A) LENGTH: 11464 base pairs
	(B) TYPE: nucleic acid
	(C) STRANDEDNESS: double
	(D) TOPOLOGY: linear
40	(ii) MOLECULE TYPE: genomic DNA
	(vi) ORIGINAL SOURCE:
	(A) ORGANISM: human
	(G) CELL TYPE: placenta
45	(ix) FEATURE:
	(A) NAME/KEY: 5' UTR
	(B) LOCATION: 1..3
	(C) IDENTIFICATION METHOD: E
	(A) NAME/KEY: leader peptide
	(B) LOCATION: 4..82
50	(C) IDENTIFICATION METHOD: S
	(A) NAME/KEY: intron
	(B) LOCATION: 83..1453
	(C) IDENTIFICATION METHOD: E
	(A) NAME/KEY: leader peptide
55	(B) LOCATION: 1454..1465
	(C) IDENTIFICATION METHOD: S

(A) NAME/KEY: intron  
 (B) LOCATION: 1466..4848  
 (C) IDENTIFICATION METHOD: E  
 (A) NAME/KEY: leader peptide  
 (B) LOCATION: 4849..4865  
 (C) IDENTIFICATION METHOD: S  
 (A) NAME/KEY: mat peptide  
 (B) LOCATION: 4866..4983  
 (C) IDENTIFICATION METHOD: S  
 (A) NAME/KEY: intron  
 (B) LOCATION: 4984..6317  
 (C) IDENTIFICATION METHOD: E  
 (A) NAME/KEY: mat peptide  
 (B) LOCATION: 6318..6451  
 (C) IDENTIFICATION METHOD: S  
 (A) NAME/KEY: intron  
 (B) LOCATION: 6452..11224  
 (C) IDENTIFICATION METHOD: E  
 (A) NAME/KEY: mat peptide  
 (B) LOCATION: 11225..11443  
 (C) IDENTIFICATION METHOD: S  
 (A) NAME/KEY: 3' UTR  
 (B) LOCATION: 11444..11464  
 (C) IDENTIFICATION METHOD: E

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 17:

25	AAG ATG GCT GCT GAA CCA GTA GAA GAC AAT TGC ATC AAC TTT GTG GCA	48
	Met Ala Ala Glu Pro Val Glu Asp Asn Cys Ile Asn Phe Val Ala	
	-35 -30 -25	
	ATG AAA TTT ATT GAC AAT ACG CTT TAC TTT ATA G GTAAGG CTAATGCCAT	98
	Met Lys Phe Ile Asp Asn Thr Leu Tyr Phe Ile Ala	
30	-20 -15 -10	
	AGAACAAATA CCAGGTTTCAG ATAAATCTAT TCAATTAGAA AAGATGTTGT GAGGTGAACT	158
	ATTAAGTGAC TCTTTGTGTC ACCAAATTTT ACTGTAATAT TAATGGCTCT TAAAAAATA	218
	GTGGACCTCT AGAAATTAAC CACAACATGT CCAAGGTCTC AGCACCTTGT CACACCACGT	278
	GTCCTGGCAC TTTAATCAGC AGTAGCTCAC TCTCCAGTTG GCAGTAAGTG CACATCATGA	338
	AAATCCCAGT TTTCATGGGA AAATCCCAGT TTTCATTGGA TTTCATGGG AAAAATCCCA	398
35	GTACAAAAC TGGTGCATTC AGGAAATACA ATTTCCCAAA GCAAATTGGC AAATTATGTA	458
	AGAGATTCTC TAAATTTAGA GTTCCGTGAA TTACACCATT TTATGTAAAT ATGTTTGACA	518
	AGTAAAAATT GATTCTTTTT TTTTTTTTCT GTTGCCCAGG CTGGAGTGCA GTGGCACAAT	578
	CTCTGCTCAC TGCAACCTCC ACCTCCTGGG TTCAAGCAAT TCTCCTGCCT CAGCCTTCTG	638
	AGTAGCTGGG ACTACAGGTG CATCCCGCCA TGCCTGGCTA ATTTTTGGGT ATTTTTACTA	698
40	GAGACAGGGT TTTGGCATGT TGCTGGGATT ACAGGCATGA ACCACCACAC ATGGCCTAAA	758
	CCTGGCTCGG GCTCCCAAAG TGCTGGGATT ACAGGCATGA ACCACCACAC ATGGCCTAAA	818
	AATTGATTCT TATGATTAAT CTCCTGTGAA CAATTTGGCT TCATTTGAAA GTTTGCCTTC	878
	ATTTGAAACC TTCATTTAAA AGCCTGAGCA ACAAAGTGAG ACCCCATCTC TACAAAAAAC	938
	TGCAAAATAT CCTGTGGACA CCTCCTACCT TCTGTGGAGG CTGAAGCAGG AGGATCACTT	998
	GAGCCTAGGA ATTTGAGCCT GCAGTGAGCT ATGATCCAC CCCTACACTC CAGCCTGCAT	1058
45	GACAGTAGAC CCTGACACAC ACACACAAAA AAAAACCTTC ATAAAAAATT ATTAGTTGAC	1118
	TTTTCTTAGG TGACTTTCCG TTAAAGCAAT AAATTTAAAA GTAAATCTC TAATTTTAGA	1178
	AAATTTATTT TTAGTTACAT ATTGAAATTT TTAACCCTA GGTTTAAGTT TTATGTCTAA	1238
	ATTACCTGAG AACACACTAA GTCTGATAAG CTTCAATTTA TGGGCCTTTT GGATGATTAT	1298
	ATAATATTCT GATGAAAGCC AAGACAGACC CTTAAACCAT AAAAATAGGA GTTCGAGAAA	1358
50	GAGGAGTAGC AAAAGTAAAA GCTAGAATGA GATTGAATTC TGAGTCGAAA TACAAAATTT	1418
	TACATATTCT GTTTCTCTCT TTTTCCCCCT CTTAG CT GAA GAT GAT G GTAAA	1470
	Ala Glu Asp Asp Glu	
	-10	
	GTAGAAATGA ATTTATTTTT CTTTGCAAAC TAAGTATCTG CTTGAGACAC ATCTATCTCA	1530
	CCATTGTCAG CTGAGGAAAA AAAAAAATGG TTCTCATGCT ACCAATCTGC CTTCAAAGAA	1590
55	ATGTGGACTC AGTAGCACAG CTTTGGAATG AAGATGATCA TAAGAGATAC AAAGAAGAAC	1650
	CTCTAGCAAA AGATGCTTCT CTATGCCTTA AAAAATTCTC CAGCTCTTAG AATCTACAAA	1710

	ATAGACTTTG	CCTGTTTCAT	TGGTCCTAAG	ATTAGCATGA	AGCCATGGAT	TCTGTTGTAG	1770
	GGGGAGCGTT	GCATAGGAAA	AAGGGATTGA	AGCATTAGAA	TTGTCCAAAA	TCAGTAACAC	1830
	CTCCTCTCAG	AAATGCTTTG	GGAAGAAGCC	TGGAAGGTTT	CGGGTTGGTG	GTGGGGTGGG	1890
5	GCAGAAAATT	CTGGAAGTAG	AGGAGATAGG	AATGGGTGGG	GCAAGAAGAC	CACATTCAGA	1950
	GGCCAAAAGC	TGAAAGAAAC	CATGGCATT	ATGATGAATT	CAGGGTAATT	CAGAATGGAA	2010
	GTAGAGTAGG	AGTAGGAGAC	TGGTGAGAGG	AGCTAGAGTG	ATAAACAGGG	TGTAGAGCAA	2070
	GACGTTCTCT	CACCCCAAGA	TGTGAAATTT	GGACTTTATC	TTGGAGATAA	TAGGGTTAAT	2130
	TAAGCACAAT	ATGTATTAGC	TAGGGTAAAG	ATTAGTTTGT	TGTAACAAAG	ACATCCAAAG	2190
	ATACAGTAGC	TGAATAAGAT	AGAGAATTTT	TCTCTCAAAG	AAAGTCTAAG	TAGGCAGCTC	2250
10	AGAAGTAGTA	TGGCTGGAAG	CAACCTGATG	ATATTGGGAC	CCCCAACCTT	CTTCAGTCTT	2310
	GTACCCATCA	TCCCCTAGTT	GTTGATCTCA	CTCACATAGT	TGAAAATCAT	CATACTTCCT	2370
	GGGTTCATAT	CCCAGTTATC	AAGAAAGGGT	CAAGAGAAGT	CAGGCTCATT	CCTTTCAAAG	2430
	ACTCTAATTG	GAAGTTAAAC	ACATCAATCC	CCCTCATATT	CCATTGACTA	GAATTTAATC	2490
	ACATGGCCAC	ACCAAGTGCA	AGGAAATCTG	GAAAATATAA	TCTTTATTCC	AGGTAGCCAT	2550
	ATGACTCTTT	AAAATTCAGA	AATAATATAT	TTTTAAATAA	TCATTCTGGC	TTTGGTATAA	2610
15	AGAATTGATG	GTGTGGGGTG	AGGAGGCCAA	AATTAAGGGT	TGAGAGCCTA	TTATTTTAGT	2670
	TATTACAAGA	AATGATGGTG	TCATGAATTA	AGGTAGACAT	AGGGGAGTGC	TGATGAGGAG	2730
	CTGTGAATGG	ATTTTAGAAA	CACTTGAGAG	AATCAATAGG	ACATGATTTA	GGGTTGGATT	2790
	TGGAAAGGAG	AAGAAAGTAG	AAAAGATGAT	GCCTACATTT	TTCACTTAGG	CAATTTGTAC	2850
	CATTCAGTGA	AATAGGGAAC	ACAGGAGGAA	GAGCAGGTTT	TGGTGTATAC	AAAGAGGAGG	2910
20	ATGGATGACG	CATTTTCGTTT	TGGATCTGAG	ATGTCTGTGG	AACGTCCTAG	TGGAGATGTC	2970
	CACAAACTCT	TCTACATGTG	GTTCTGAGTT	CAGGACACAG	ATTTGGGCTG	GAGATAGAGA	3030
	TATTGTAGGC	TTATACATAG	AAATGGCATT	TGAATCTATA	GAGATAAAAA	GACACATCAG	3090
	AGGAAATGTG	TAAAGTGAGA	GAGGAAAAGC	CAAGTACTGT	GCTGGGGGGA	ATACCTACAT	3150
	TTAAAGGATG	CAGTAGAAAG	AAGCTAATAA	ACAACAGAGA	GCAGACTAAC	CAAAAGGGGA	3210
	GAAGAAAAAC	CAAGAGAATT	CCACCGACTC	CCAGGAGAGC	ATTTCAAGAT	TGAGGGGATA	3270
25	GGTGTGTGTG	TGAATTTTGC	AGCCTTGAGA	ATCAAGGGCC	AGAACACAGC	TTTTAGATTT	3330
	AGCAACAAGG	AGTTTGGTGA	TCTCAGTGAA	AGCAGCTTGA	TGGTGAAATG	GAGGCAGAGG	3390
	CAGATTGCAA	TGAGTGAAAC	AGTGAATGGG	AAGTGAAGAA	ATGATACAGA	TAATTCCTTG	3450
	TAAAAGCTTG	GCTGTAAAAA	GGAGGAGAGA	AACAAGACTA	GCTGCAAAGT	GAGATTGGGT	3510
	TGATGGAGCA	GTTTTAAATC	TCAAAATAAA	GAGCTTTGTG	CTTTTTTTGAT	TATGAAAATA	3570
	ATGTGTAAAT	TGTAACATAA	TGAGGCAATG	AAAAAAGATA	ATAATATGAA	AGATAAAAAAT	3630
30	ATAAAAACCA	CCCAGAAATA	ATGATAGCTA	CCATTTTGTG	ACAATATTTT	TACACTCCTT	3690
	TCTATGTATA	TATACAGACA	CAGAAATGCT	TATATTTTTA	TTAAAAGGGA	TTGTACTATA	3750
	CCTAAGCTGC	TTTTTCTAGT	TAGTGATATA	TATGGACATC	TCTCCATGGC	AACGAGTAAT	3810
	TGCAGTTATA	TTAAGTTCAT	GATATTTTAC	AATAAGGGCA	TATCTTTGCC	CTTTTTTATTT	3870
	AATCAATTCT	TAATTGGTGA	ATGTTTGTG	CCAGTTTGTG	GTTGTTATTA	ACAATGTTCC	3930
35	CATAAGCATT	CCTGTACACC	AATGTTTACA	CATTTGTCTG	ATTTTTTCTT	CAGGATAAAA	3990
	CCCAGGAGGT	AGAATTGCTG	GGTTGATAGA	AGAGAAAGGA	TGATTGCCAA	ATTAAAGCTT	4050
	CAGTAGAGGG	TACATGCCGA	GCACAAATGG	GATCAGCCCT	AGATACCAGA	AATGGCACTT	4110
	TCTCATTTCC	CCTTGGGACA	AAAGGGAGAG	AGGCAATAAC	TGTGCTGCCA	GAGTTAAATT	4170
	TGTACGTGGA	GTAGCAGGAA	ATCATTTGCT	GAAAATGAAA	ACAGAGATGA	TGTTGTAGAG	4230
	GTCCTGAAGA	GAGCAAAGAA	AATTTGAAAT	TGCGGCTATC	AGCTATGGAA	GAGAGTGCTG	4290
40	AACTGGAAAA	CAAAAGAAGT	ATTGACAATT	GGTATGCTTG	TAATGGCACC	GATTTGAACG	4350
	CTTGTGCCAT	TGTTTACCAG	CAGCACTCAG	CAGCCAAGTT	TGGAGTTTTG	TAGCAGAAAG	4410
	ACAAATAAGT	TAGGGATTTA	ATATCCTGGC	CAAATGGTAG	ACAAAATGAA	CTCTGAGATC	4470
	CAGCTGCACA	GGGAAGGAAG	GGAAGACGGG	AAGAGGTTAG	ATAGGAAATA	CAAGAGTCAG	4530
	GAGACTGGAA	GATGTTGTGA	TATTTAAGAA	CACATAGAGT	TGGAGTAAAA	GTGTAAGAAA	4590
	ACTAGAAGGG	TAAGAGACCG	GTCAGAAAGT	AGGCTATTTG	AAGTTAACAC	TTCAGAGGCA	4650
45	GAGTAGTTCT	GAATGGTAAC	AAGAAATTGA	GTGTGCCTTT	GAGAGTAGGT	TAAAAACAA	4710
	TAGGCAACTT	TATTGTAGCT	ACTTCTGGAA	CAGAAGATTG	TCATTAATAG	TTTTAGAAAA	4770
	CTAAAATATA	TAGCATACTT	ATTTGTCAAT	TAACAAAGAA	ACTATGTATT	TTTAAATGAG	4830
	ATTTAATGTT	TATTGTAG	AA AAC CTG GAA TCA GAT	TAC TTT GGC AAG CTT			4880
			Glu Asn Leu Glu Ser Asp	Tyr Phe Gly Lys Leu			
			-5	1		5	
50	GAA TCT AAA TTA TCA GTC ATA AGA AAT TTG AAT	GAC CAA GTT CTC TTC					4928
	Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn	Asp Gln Val Leu Phe					
			10	15		20	
	ATT GAC CAA GGA AAT CGG CCT CTA TTT GAA GAT	ATG ACT GAT TCT GAC					4976
	Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp	Met Thr Asp Ser Asp					
			25	30		35	
55	TGT AGA G GTATTTTTT TTAATTCGCA AACATAGAAA	TGACTAGCTA CTTCTTCCCA					5032

Cys Arg Asp

40

5	TTCTGTTTTA	CTGCTTACAT	TGTTCCGTGC	TAGTCCCAAT	CCTCAGATGA	AAAGTCACAG	5092
	GAGTGACAAT	AATTTCACTT	ACAGGAAACT	TTATAAGGCA	TCCACGTTTT	TTAGTTGGGG	5152
	TAAAAAATTG	GATACAATAA	GACATTGCTA	GGGGTCATGC	CTCTCTGAGC	CTGCCTTTGA	5212
	ATCACCAATC	CCTTTATTGT	GATTGCATTA	ACTGTTTAAA	ACCTCTATAG	TTGGATGCTT	5272
	AATCCCTGCT	TGTTACAGCT	GAAAATGCTG	ATAGTTTACC	AGGTGTGGTG	GCATCTATCT	5332
	GTAATCCTAG	CTACTTGGGA	GGCTCAAGCA	GGAGGATTGC	TTGAGGCCAG	GACTTTGAGG	5392
	CTGTAGTACA	CTGTGATCGT	ACCTGTGAAT	AGCCACTGCA	CTCCAGCCTG	GGTGATATAC	5452
10	AGACCTTGTC	TCTAAAATTA	AAAAAAAAAA	AAAAAAAAAC	CTTAGGAAAG	GAAATTGATC	5512
	AAGTCTACTG	TGCCTTCCAA	AACATGAATT	CCAAATATCA	AAGTTAGGCT	GAGTTGAAGC	5572
	AGTGAATGTG	CATTCTTTAA	AAATACTGAA	TACTTACCTT	AACATATATT	TTAAATATTT	5632
	TATTTAGCAT	TTAAAAGTTA	AAAACAATCT	TTTAGAATTC	ATATCTTTAA	AATACTCAAA	5692
	AAAGTTGCAG	CGTGTGTGTT	GTAATACACA	TTAAACTGTG	GGGTTGTTTG	TTTGTTTGAG	5752
15	ATGCAGTTTC	ACTCTGTCAC	CCAGGCTGAA	GTGCAGTGCA	GTGCAGTGGT	GTGATCTCGG	5812
	CTCACTACAA	CCTCCACCTC	CCACGTTCAA	GCGATTCTCA	TGCCTCAGTC	TCCCGAGTAG	5872
	GTGGGATTAC	AGGCATGCAC	CACTTACACC	CGGCTAATTT	TTGTATTTTT	AGTAGAGCTG	5932
	GGGTTTCACC	ATGTTGGCCA	GGCTGGTCTC	AAACCCCTAA	CCTCAAGTGA	TCTGCCTGCC	5992
	TCAGCCTCCC	AAACAAACAA	ACAACCCAC	AGTTTAATAT	GTGTTACAAC	ACACATGCTG	6052
	CAACTTTTAT	GAGTATTTTA	ATGATATAGA	TTATAAAAGG	TTGTTTTTAA	CTTTTAAATG	6112
20	CTGGGATTAC	AGGCATGAGC	CACTGTGCCA	GGCCTGAACT	GTGTTTTTAA	AAATGTCTGA	6172
	CCAGCTGTAC	ATAGTCTCCT	GCAGACTGGC	CAAGTCTCAA	AGTGGGAACA	GGTGTATTAA	6232
	GGACTATCCT	TTGGTTAAAT	TTCCGCAAAT	GTTCTGTGTC	AAGAATTCTT	CTAACTAGAG	6292
	TTCTCATTTA	TTATATTTAT	TTCAG	AT AAT GCA CCC	CGG ACC ATA TTT ATT		6343
			Asp Asn Ala Pro	40	Arg Thr Ile Phe Ile		
25	ATA AGT ATG TAT AAA GAT AGC CAG CCT AGA GGT	ATG GCT GTA ACT ATC					6391
	Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly	Met Ala Val Thr Ile					
	50	55	60				
	TCT GTG AAG TGT GAG AAA ATT TCA ACT CTC TCC	TGT GAG AAC AAA ATT					6439
	Ser Val Lys Cys Glu Lys Ile Ser Thr Leu Ser	Cys Glu Asn Lys Ile					
	65	70	75	80			
30	ATT TCC TTT AAG GTAAG ACTGAGCCTT ACTTTGTTTT	CAATCATGTT AATATAATCA					6496
	Ile Ser Phe Lys						
	ATATAATTAG AAATATAACA TTATTTCTAA TGTTAATATA AGTAATGTAA TTAGAAAAC						6556
	CAAATATCCT CAGACCAACC TTTTGTCTAG AACAGAAATA ACAAGAAGCA GAGAACCATT						6616
	AAAGTGAATA CTTACTAAAA ATTATCAAAC TCTTTACCTA TTGTGATAAT GATGGTTTTT						6676
35	CTGAGCCTGT CACAGGGGAA GAGGAGATAC AACACTTGTT TTATGACCTG CATCTCCTGA						6736
	ACAATCAGTC TTTATACAAA TAATAATGTA GAATACATAT GTGAGTTATA CATTTAAGAA						6796
	TAACATGTGA CTTTCCAGAA TGAGTTCTGC TATGAAGAAT GAAGCTAATT ATCCTTCTAT						6856
	ATTTCTACAC CTTTGTAAT TATGATAATA TTTTAATCCC TAGTTGTTTT GTTGCTGATC						6916
	CTTAGCCTAA GTCTTAGACA CAAGCTTCAG CTTCCAGTTG ATGTATGTTA TTTTAAATGT						6976
	TAATCTAATT GAATAAAAGT TATGAGATCA GCTGTAAAAG TAATGCTATA ATTATCTTCA						7036
40	AGCCAGGTAT AAAGTATTTT TGGCCTCTAC TTTTCTCTA TTATTCTCCA TTATTATTCT						7096
	CTATTATTTT TCTCTATTTT CTCCATTATT GTTAGATAAA CCACAATTAA CTATAGCTAC						7156
	AGACTGAGCC AGTAAGAGTA GCCAGGGATG CTTACAAATT GGCAATGCTT CAGAGGAGAA						7216
	TTCCATGTCA TGAAGACTCT TTTTGAGTGG AGATTTGCCA ATAAATATCC GCTTTCATGC						7276
	CCACCCAGTC CCCACTGAAA GACAGTTAGG ATATGACCTT AGTGAAGGTA CCAAGGGGCA						7336
	ACTTGGTAGG GAGAAAAAAG CCACTCTAAA ATATAATCCA AGTAAGAACA GTGCATATGC						7396
45	AACAGATACA GCCCCCAGAC AAATCCCTCA GCTATCTCCC TCCAACCAGA GTGCCACCCC						7456
	TTCAGGTGAC AATTTGGAGT CCCCATTTCTA GACCTGACAG GCAGCTTAGT TATCAAAATA						7516
	GCATAAGAGG CCTGGGATGG AAGGGTAGGG TGGAAAGGGT TAAGCATGCT GTTACTGAAC						7576
	AACATAATTA GAAGGGAAGG AGATGGCCAA GCTCAAGCTA TGTGGGATAG AGGAAAAC						7636
	AGCTGCAGAG GCAGATTCAG AAAGTGGGAT AAGTCCGAAC CTACAGGTGG ATTCTTGTTG						7696
50	AGGGAGACTG GTGAAAATGT TAAGAAGATG GAAATAATGC TTGGCACTTA GTAGGAACTG						7756
	GGCAAATCCA TATTTGGGGG AGCCTGAAGT TTATTCAATT TTGATGGCCC TTTTAAATAA						7816
	AAAGAATGTG GCTGGGCGTG GTGGCTCACA CCTGTAATCC CAGCACTTTG GGAGGCCGAG						7876
	GGGGGCGGAT CACCTGAAGT CAGGAGTTCA AGACCAGCCT GACCAACATG GAGAAACCCC						7936
	ATCTCTACTA AAAATACAAA ATTAGCTGGG CGTGGTGGCA TATGCCTGTA ATCCCAGCTA						7996
	CTCGGGAGGC TGAGGCAGGA GAATCTTTTG AACCCGGGAG GCAGAGGTTG CGATGAGCCT						8056
55	AGATCGTGCC ATTGCACTCC AGCCTGGGCA ACAAGAGCAA AACTCGGTCT CAAAAA						8116
	AAAAAAAAG TGAAATTAAC CAAAGGCATT AGCTTAATAA TTTAATACTG TTTTAAAGTA						8176

	GGGCGGGGGG	TGGCTGGAAG	AGATCTGTGT	AAATGAGGGA	ATCTGACATT	TAAGCTTCAT	8236
	CAGCATCATA	GCAAATCTGC	TTCTGGAAGG	AACTCAATAA	ATATTAGTTG	GAGGGGGGGA	8296
	GAGAGTGAGG	GGTGGACTAG	GACCAGTTTT	AGCCCTTGTC	TTTAATCCCT	TTTCCTGCCA	8356
5	CTAATAAGGA	TCTTAGCAGT	GGTTATAAAA	GTGGCCTAGG	TTCTAGATAA	TAAGATACAA	8416
	CAGGCCAGGC	ACAGTGGCTC	ATGCCTATAA	TCCCAGCACT	TTGGGAGGGC	AAGGCGAGTG	8476
	TCTCACTTGA	GATCAGGAGT	TCAAGACCAG	CCTGGCCAGC	ATGGCGATAC	TCTGTCTCTA	8536
	CTAAAAAATA	TACAAAAATT	AGCCAGGCAT	GGTGGCATGC	ACCTGTAATC	CCAGCTACTC	8596
	GTGAGCCTGA	GGCAGAAGAA	TCGCTTGAAA	CCAGGAGGTG	TAGGCTGCAG	TGAGCTGAGA	8656
	TCGCACCACT	GCACTCCAGC	CTGGGCGACA	GAATGAGACT	TTGTCTCAAA	AAAAGAAAAA	8716
10	GATACAACAG	GCTACCCTTA	TGTGCTCACC	TTTCACTGTT	GATTACTAGC	TATAAAGTCC	8776
	TATAAAGTTC	TTTGGTCAAG	AACCTTGACA	ACACTAAGAG	GGATTTGCTT	TGAGAGGTTA	8836
	CTGTCAGAGT	CTGTTTCATA	TATATACATA	TACATGTATA	TATGTATCTA	TATCCAGGCT	8896
	TGGCCAGGGT	TCCCTCAGAC	TTTCCAGTGC	ACTTGGGAGA	TGTTAGGTCA	ATATCAACTT	8956
	TCCCTGGATT	CAGATTCAAC	CCCTTCTGAT	GTAAAAAATA	AAAAAATAAA	GAAAGAAATC	9016
	CCTTTCCCCT	TGGAGCACTC	AAGTTTCACC	AGGTGGGGCT	TTCCAAGTTG	GGGGTTCTCC	9076
15	AAGGTCATTG	GGATTGCTTT	CACATCCATT	TGCTATGTAC	CTTCCCTATG	ATGGCTGGGA	9136
	GTGGTCAACA	TCAAACTAG	GAAAGCTACT	GCCCAAGGAT	GTCCTTACCT	CTATTCTGAA	9196
	ATGTGCAATA	AGTGTGATTA	AAGAGATTGC	CTGTTCTACC	TATCCACACT	CTCGCTTTCA	9256
	ACTGTAACCT	TCTTTTTTTC	TTTTTTTCTT	TTTTTCTTTT	TTTTTGAAAC	GGAGTCTCGC	9316
	TCTGTGCGCC	AGGCTAGAGT	GCAGTGGCAC	GATCTCAGCT	CACTGCAAGC	TCTGCCTCCC	9376
20	GGGTTACACG	CATTCTCCTG	CCTCACCTTC	CCAAGCAGCT	GGGACTACAG	GCGCCTGCCA	9436
	CCATGCCCAG	CTAATTTTTT	GTATTTTTAG	TAGAGACGGG	GTTTCACCGT	GTTAGCCAGG	9496
	ATGGTCTCGA	TCTCCTGAAC	TTGTGATCCG	CCCGCCTCAG	CCTCCCAAAG	TGCTGGGATT	9556
	ACAGGCGTGA	GCCATCGCAC	CCGGCTCAAC	TGTAACCTTC	TATACTGGTT	CATCTTCCCC	9616
	TGTAATGTTA	CTAGAGCTTT	TGAAGTTTTG	GCTATGGATT	ATTTCTCATT	TATACATTAG	9676
	ATTTTCAGATT	AGTTCCAAAT	TGATGCCAC	AGCTTAGGGT	CTCTTCCTAA	ATTGTATATT	9736
25	GTAGACAGCT	GCAGAAGTGG	GTGCCAATAG	GGGAAC TAGT	TTATACTTTC	ATCAACTTAG	9796
	GACCCACACT	TGTTGATAAA	GAACAAAGGT	CAAGAGTTAT	GACTACTGAT	TCCACAAC TG	9856
	ATTGAGAAGT	TGGAGATAAC	CCCGTGACCT	CTGCCATCCA	GAGTCTTTCA	GGCATCTTTG	9916
	AAGGATGAAG	AAATGCTATT	TTAATTTTGG	AGGTTTCTCT	ATCAGTGCTT	AGGATCATGG	9976
	GAATCTGTGC	TGCCATGAGG	CCAAAATTAA	GTCCAAAACA	TCTACTGGTT	CCAGGATTAA	10036
30	CATGGAAGAA	CCTTAGGTGG	TGCCCACATG	TTCTGATCCA	TCCTGCAAAA	TAGACATGCT	10096
	GCACTAACAG	GAAAAGTGCA	GGCAGCACTA	CCAGTTGGAT	AACCTGCAAG	ATTATAGTTT	10156
	CAAGTAATCT	AACCATTCTT	CACAAGGCCC	TATTCTGTGA	CTGAAACATA	CAAGAATCTG	10216
	CATTTGGCCT	TCTAAGGCAG	GGCCCAGCCA	AGGAGACCAT	ATTCAGGACA	GAAATTCAAG	10276
	ACTACTATGG	AACTGGAGTG	CTTGGCAGGG	AAGACAGAGT	CAAGGACTGC	CAACTGAGCC	10336
	AATACAGCAG	GCTTACACAG	GAACCCAGGG	CCTAGCCCTA	CAACAATTAT	TGGGTCTATT	10396
35	CACTGTAAGT	TTTAATTTCA	GGCTCCACTG	AAAGAGTAAG	CTAAGATTCC	TGGCACTTTT	10456
	TGTCTCTCTC	ACAGTTGGCT	CAGAAATGAG	AACTGGTCAG	GCCAGGCATG	GTGGCTTACA	10516
	CCTGGAATCC	CAGCACTTTG	GGAGGCCGAA	GTGGGAGGGT	CACTTGAGGC	CAGGAGTTCA	10576
	GGACCAGCTT	AGGCAACAAA	GTGAGATACC	CCCTGACCCC	TTCTCTACAA	AAATAAATTT	10636
	TAAAAATTAG	CCAAATGTGG	TGGTGTATAC	TTACAGTCCC	AGCTACTCAG	GAGGCTGAGG	10696
	CAGGGGGATT	GCTTGAGCCC	AGGAATTCAA	GGCTGCAGTG	AGCTATGATT	TCACCACTGC	10756
40	ACTTCTGGCT	GGGCAACAGA	GCGAGACCCT	GTCTCAAAGC	AAAAAGAAAA	AGAAACTAGA	10816
	ACTAGCCTAA	GTTTGTGGGA	GGAGGTCATC	ATCGTCTTTA	GCCGTGAATG	GTTATTATAG	10876
	AGGACAGAAA	TTGACATTAG	CCCAAAAAGC	TTGTGGTCTT	TGCTGGAACT	CTACTTAATC	10936
	TTGAGCAAAT	GTGGACACCA	CTCAATGGGA	GAGGAGAGAA	GTAAGCTGTT	TGATGTATAG	10996
	GGGAAAATA	GAGGCCTGGA	ACTGAATATG	CATCCCATGA	CAGGGAGAAT	AGGAGATTCT	11056
45	GAGTTAAGAA	GGAGAGGAGG	TCAGTACTGC	TGTTTCAGAGA	TTTTTTTTTAT	GTAACCTCTT	11116
	AGAAGCAAAA	CTACTTTTGT	TCTGTTTGGT	AATATACTTC	AAAACAAACT	TCATATATTC	11176
	AAATTGTTCA	TGTCCTGAAA	TAATTAGGTA	ATGTTTTTTT	CTCTATAG	GAA ATG AAT	11233
						Glu Met Asn	
						85	
50	CCT CCT GAT AAC ATC AAG GAT ACA AAA AGT GAC	ATC ATA TTC TTT CAG	11281				
	Pro Pro Asp Asn Ile Lys Asp Thr Lys Ser Asp	Ile Ile Phe Phe Glu					
	90	95	100				
	AGA AGT GTC CCA GGA CAT GAT AAT AAG ATG CAA	TTT GAA TCT TCA TCA	11329				
	Arg Ser Val Pro Gly His Asp Asn Lys Met Gln	Phe Glu Ser Ser Ser					
	105	110	115				
55	TAC GAA GGA TAC TTT CTA GCT TGT GAA AAA GAG	AGA GAC CTT TTT AAA	11377				
	Tyr Glu Gly Tyr Phe Leu Ala Cys Glu Lys Glu	Arg Asp Leu Phe Lys					
	120	125	130				135

CTC ATT TTG AAA AAA GAG GAT GAA TTG GGG GAT AGA TCT ATA ATG-TTC 11425  
 Leu Ile Leu Lys Lys Glu Asp Glu Leu Gly Asp Arg Ser Ile Met Phe  
                   140                  145                  150  
 5 ACT GTT CAA AAC GAA GAC TAGCTATTAA AATTTCATGC C 11464  
 Thr Val Gln Asn Glu Asp  
                   155

## (19) INFORMATION FOR SEQ ID NO: 18:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 471 base pairs  
 (B) TYPE: nucleic acid  
 (C) STRANDEDNESS: double  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: cDNA to mRNA

(vi) ORIGINAL SOURCE:

(A) ORGANISM: mouse  
 (G) CELL TYPE: liver

(ix) FEATURE:

(A) NAME/KEY: mat peptide  
 (B) LOCATION: 1..471  
 (C) IDENTIFICATION METHOD: S

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 18:

AAC TTT GGC CGA CTT CAC TGT ACA ACC GCA GTA ATA CGG AAT ATA AAT 48  
 Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn  
 1                  5                  10                  15  
 30 GAC CAA GTT CTC TTC GTT GAC AAA AGA CAG CCT GTG TTC GAG GAT ATG 96  
 Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met  
                   20                  25                  30  
 ACT GAT ATT GAT CAA AGT GCC AGT GAA CCC CAG ACC AGA CTG ATA ATA 144  
 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile  
                   35                  40                  45  
 35 TAC ATG TAC AAA GAC AGT GAA GTA AGA GGA CTG GCT GTG ACC CTC TCT 192  
 Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser  
                   50                  55                  60  
 GTG AAG GAT AGT AAA ATG TCT ACC CTC TCC TGT AAG AAC AAG ATC ATT 240  
 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile  
                   65                  70                  75                  80  
 40 TCC TTT GAG GAA ATG GAT CCA CCT GAA AAT ATT GAT GAT ATA CAA AGT 288  
 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser  
                   85                  90                  95  
 GAT CTC ATA TTC TTT CAG AAA CGT GTT CCA GGA CAC AAC AAG ATG GAG 336  
 Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu  
                   100                  105                  110  
 45 TTT GAA TCT TCA CTG TAT GAA GGA CAC TTT CTT GCT TGC CAA AAG GAA 384  
 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu  
                   115                  120                  125  
 GAT GAT GCT TTC AAA CTC ATT CTG AAA AAA AAG GAT GAA AAT GGG GAT 432  
 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp  
                   130                  135                  140  
 50 AAA TCT GTA ATG TTC ACT CTC ACT AAC TTA CAT CAA AGT 471  
 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser  
                   145                  150                  155

## (20) INFORMATION FOR SEQ ID NO: 19:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 9 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

5

(ii) MOLECULE TYPE: peptide

(v) FRAGMENT TYPE: N-terminal fragment

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 19:

10

Asn Phe Gly Arg Leu His Cys Thr Thr  
 1 5

(21) INFORMATION FOR SEQ ID NO: 20:

15

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

20

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 20:

25

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	1	5	10	15
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	20	25	30	
Met	Thr	Asp	Ser	Asp	Cys	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile	35	40	45	
Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile	50	55	60	
Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile	65	70	75	80
Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys	85	90	95	
Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys	100	105	110	
Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Cys	Glu	115	120	125	
Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu	130	135	140	
Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp				145	150	155	

40

(22) INFORMATION FOR SEQ ID NO: 21:

45

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

50

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 21:

55

Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn	1	5	10	15
Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp	20	25	30	
Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile				

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35 40 45  
 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
 50 55 60  
 5 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile  
 65 70 75 80  
 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
 85 90 95  
 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys  
 100 105 110  
 10 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu  
 115 120 125  
 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
 130 135 140  
 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 145 150 155  
 15

(23) INFORMATION FOR SEQ ID NO: 22:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 157 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 22:

25 Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn  
 1 5 10 15  
 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp  
 20 25 30  
 30 Met Thr Asp Ser Asp Cys Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile  
 35 40 45  
 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
 50 55 60  
 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Cys Glu Asn Lys Ile  
 65 70 75 80  
 35 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
 85 90 95  
 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys  
 100 105 110  
 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu  
 115 120 125  
 40 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
 130 135 140  
 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 145 150 155

(24) INFORMATION FOR SEQ ID NO: 23:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 157 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 23:

55 Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn  
 1 5 10 15  
 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp

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			20				25			30						
	Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile
			35					40					45			
5	Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile
		50					55					60				
	Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Cys	Glu	Asn	Lys	Ile
	65					70					75					80
	Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
					85					90					95	
10	Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100						105					110		
	Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu
			115					120					125			
	Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu
		130					135					140				
15	Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp			
	145					150						155				

(25) INFORMATION FOR SEQ ID NO: 24:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear  
 (ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 24:

	Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
	1				5				10					15		
	Asp	Gln	Val	Leu	Phe	Ile	Asp	Gln	Gly	Asn	Arg	Pro	Leu	Phe	Glu	Asp
			20						25				30			
30	Met	Thr	Asp	Ser	Asp	Ser	Arg	Asp	Asn	Ala	Pro	Arg	Thr	Ile	Phe	Ile
			35					40					45			
	Ile	Ser	Met	Tyr	Lys	Asp	Ser	Gln	Pro	Arg	Gly	Met	Ala	Val	Thr	Ile
		50					55					60				
	Ser	Val	Lys	Ser	Glu	Lys	Ile	Ser	Thr	Leu	Ser	Ser	Glu	Asn	Lys	Ile
	65					70					75					80
35	Ile	Ser	Phe	Lys	Glu	Met	Asn	Pro	Pro	Asp	Asn	Ile	Lys	Asp	Thr	Lys
					85					90					95	
	Ser	Asp	Ile	Ile	Phe	Phe	Gln	Arg	Ser	Val	Pro	Gly	His	Asp	Asn	Lys
			100						105					110		
	Met	Gln	Phe	Glu	Ser	Ser	Ser	Tyr	Glu	Gly	Tyr	Phe	Leu	Ala	Ser	Glu
			115					120					125			
40	Lys	Glu	Arg	Asp	Leu	Phe	Lys	Leu	Ile	Leu	Lys	Lys	Glu	Asp	Glu	Leu
		130					135					140				
	Gly	Asp	Arg	Ser	Ile	Met	Phe	Thr	Val	Gln	Asn	Glu	Asp			
	145					150						155				

(26) INFORMATION FOR SEQ ID NO: 25:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 25:

55	Tyr	Phe	Gly	Lys	Leu	Glu	Ser	Lys	Leu	Ser	Val	Ile	Arg	Asn	Leu	Asn
	1				5				10					15		

Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu-Asp  
 20 25 30  
 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile  
 35 40 45  
 5 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
 50 55 60  
 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile  
 65 70 75 80  
 10 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
 85 90 95  
 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys  
 100 105 110  
 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Cys Glu  
 115 120 125  
 15 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
 130 135 140  
 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 145 150 155

(27) INFORMATION FOR SEQ ID NO: 26:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 26:

Tyr Phe Gly Lys Leu Glu Ser Lys Leu Ser Val Ile Arg Asn Leu Asn  
 1 5 10 15  
 30 Asp Gln Val Leu Phe Ile Asp Gln Gly Asn Arg Pro Leu Phe Glu Asp  
 20 25 30  
 Met Thr Asp Ser Asp Ser Arg Asp Asn Ala Pro Arg Thr Ile Phe Ile  
 35 40 45  
 35 Ile Ser Met Tyr Lys Asp Ser Gln Pro Arg Gly Met Ala Val Thr Ile  
 50 55 60  
 Ser Val Lys Ser Glu Lys Ile Ser Thr Leu Ser Ala Glu Asn Lys Ile  
 65 70 75 80  
 Ile Ser Phe Lys Glu Met Asn Pro Pro Asp Asn Ile Lys Asp Thr Lys  
 85 90 95  
 Ser Asp Ile Ile Phe Phe Gln Arg Ser Val Pro Gly His Asp Asn Lys  
 100 105 110  
 40 Met Gln Phe Glu Ser Ser Ser Tyr Glu Gly Tyr Phe Leu Ala Ser Glu  
 115 120 125  
 Lys Glu Arg Asp Leu Phe Lys Leu Ile Leu Lys Lys Glu Asp Glu Leu  
 130 135 140  
 45 Gly Asp Arg Ser Ile Met Phe Thr Val Gln Asn Glu Asp  
 145 150 155

(28) INFORMATION FOR SEQ ID NO: 27:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 27:

Asn Phe Gly Arg Leu His Ala Thr Thr Ala Val Ile Arg Asn Ile Asn  
 1 5 10 15  
 Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met  
 20 25 30  
 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile  
 35 40 45  
 Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser  
 50 55 60  
 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile  
 65 70 75 80  
 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser  
 85 90 95  
 Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu  
 100 105 110  
 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Cys Gln Lys Glu  
 115 120 125  
 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp  
 130 135 140  
 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser  
 145 150 155

(29) INFORMATION FOR SEQ ID NO: 28:

(i) SEQUENCE CHARACTERISTICS:  
 (A) LENGTH: 157 amino acids  
 (B) TYPE: amino acid  
 (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 28:

Asn Phe Gly Arg Leu His Cys Thr Thr Ala Val Ile Arg Asn Ile Asn  
 1 5 10 15  
 Asp Gln Val Leu Phe Val Asp Lys Arg Gln Pro Val Phe Glu Asp Met  
 20 25 30  
 Thr Asp Ile Asp Gln Ser Ala Ser Glu Pro Gln Thr Arg Leu Ile Ile  
 35 40 45  
 Tyr Met Tyr Lys Asp Ser Glu Val Arg Gly Leu Ala Val Thr Leu Ser  
 50 55 60  
 Val Lys Asp Ser Lys Met Ser Thr Leu Ser Cys Lys Asn Lys Ile Ile  
 65 70 75 80  
 Ser Phe Glu Glu Met Asp Pro Pro Glu Asn Ile Asp Asp Ile Gln Ser  
 85 90 95  
 Asp Leu Ile Phe Phe Gln Lys Arg Val Pro Gly His Asn Lys Met Glu  
 100 105 110  
 Phe Glu Ser Ser Leu Tyr Glu Gly His Phe Leu Ala Ser Gln Lys Glu  
 115 120 125  
 Asp Asp Ala Phe Lys Leu Ile Leu Lys Lys Lys Asp Glu Asn Gly Asp  
 130 135 140  
 Lys Ser Val Met Phe Thr Leu Thr Asn Leu His Gln Ser  
 145 150 155

## Claims

1. An osteoclastgenic inhibitory agent, which comprises an interleukin-18 or its functional equivalent.
2. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequences of SEQ ID NO: 1, SEQ ID NO: 2, and SEQ ID NO: 3 as partial amino acid sequences.
3. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequences of SEQ ID NO: 4

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and SEQ ID NO: 5 as partial amino acid sequences.

4. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequence of SEQ ID NO: 6.
- 5 5. The inhibitory agent of claim 1, wherein said interleukin-18 is human origin.
6. The inhibitory agent of claim 1, wherein said interleukin-18 includes the amino acid sequence of SEQ ID NO: 7.
7. The inhibitory agent of claim 1, which is a therapeutic agent for osteoclast-related diseases.
- 10 8. The inhibitory agent of claim 1, which contains a protein, buffer, or saccharide as a stabilizer.
9. The inhibitory agent of claim 1, which is in the form of a liquid, paste, or solid.
- 15 10. The inhibitory agent of claim 1, which contains 0.000002-100 w/w % of said interleukin-18.
11. An inhibitory agent as defined in any preceding claim, for use as a pharmaceutical.
- 20 12. Use of an inhibitory agent as defined in any of claims 1-10 for the preparation of a medicament effective for treating and/or preventing osteoclast-related diseases.

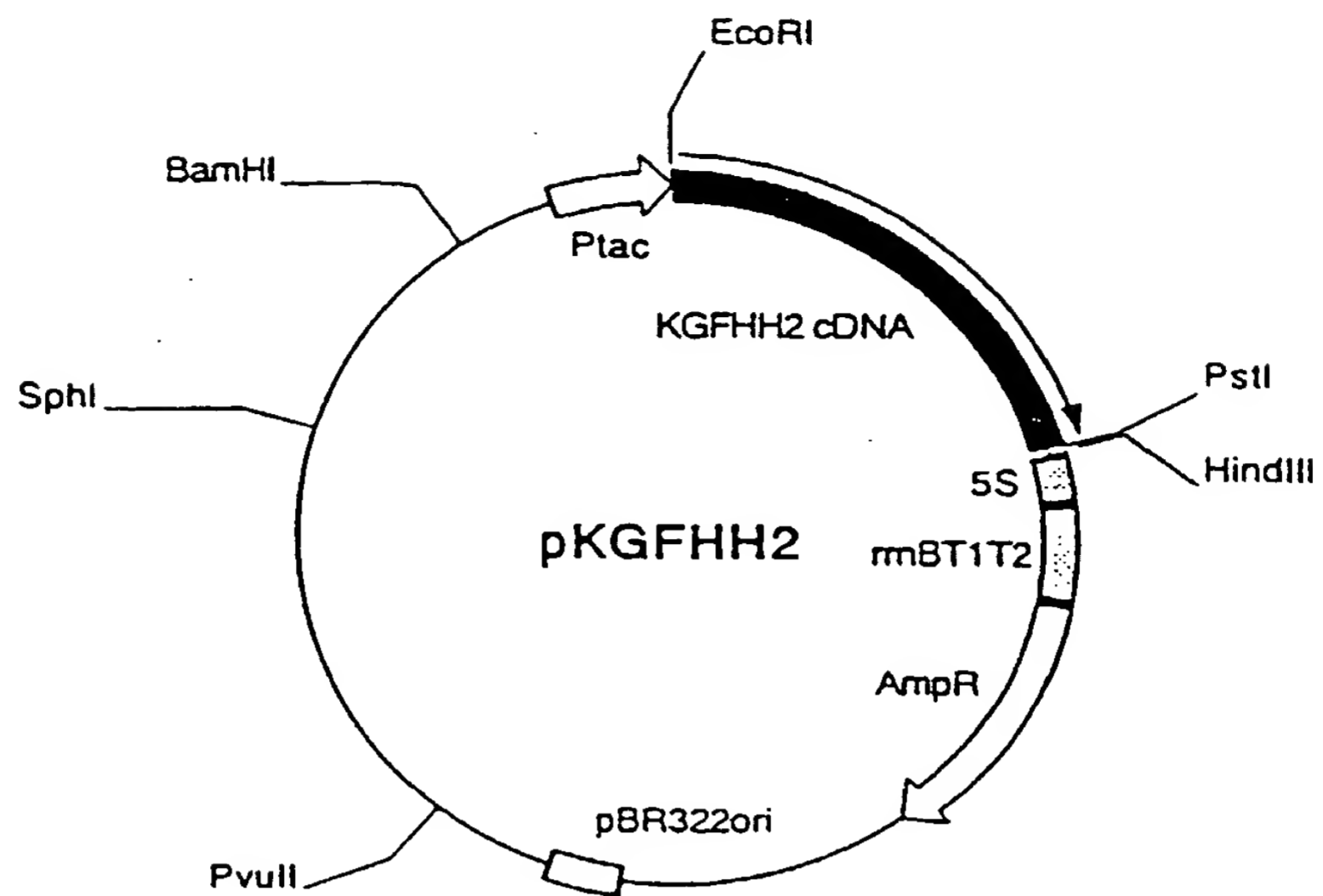


FIG. 1

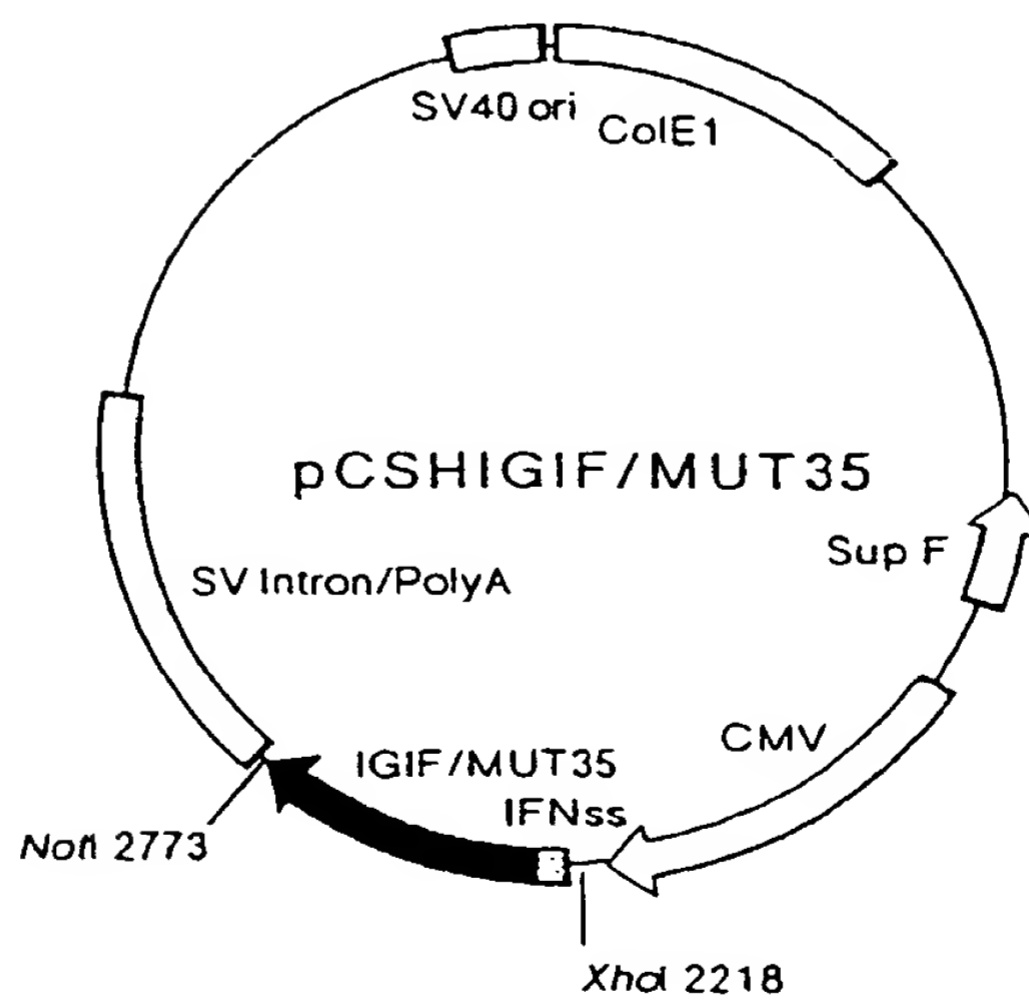
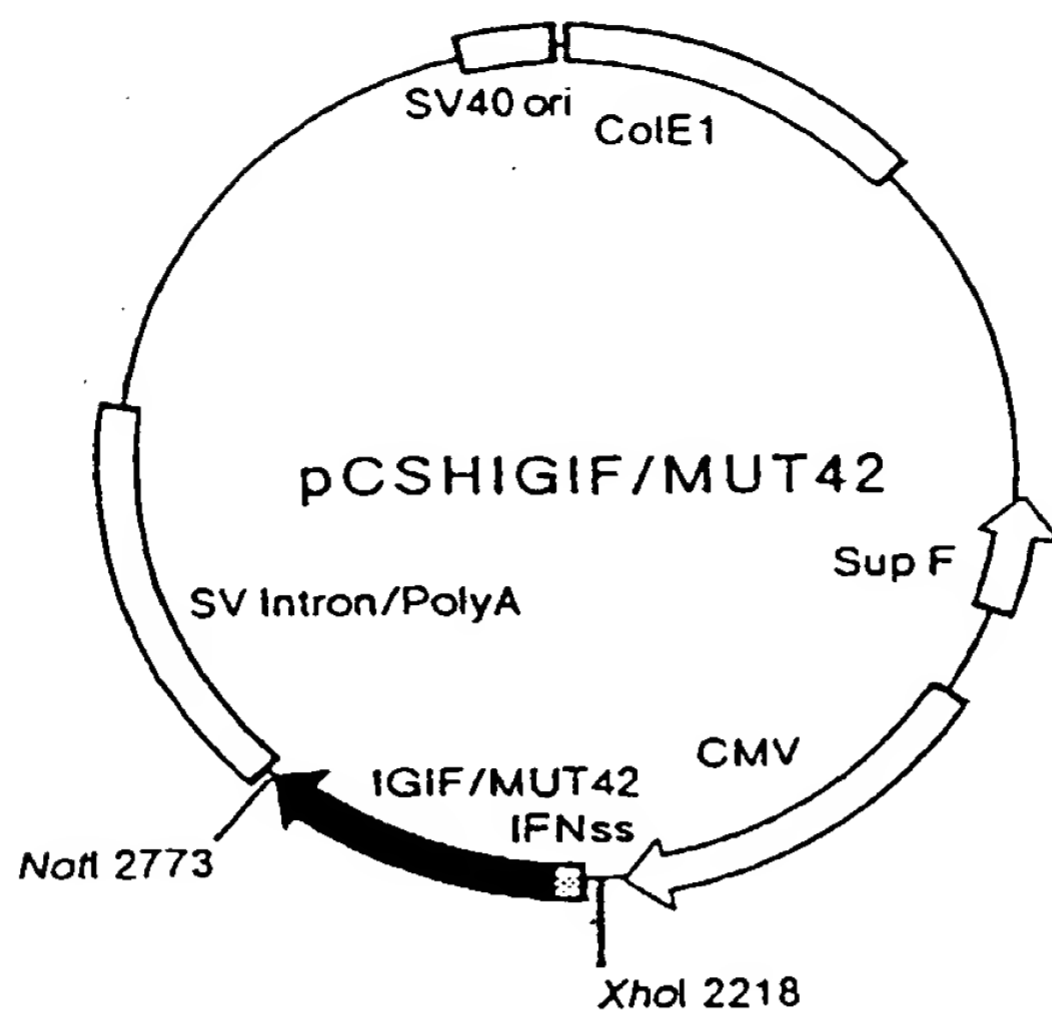
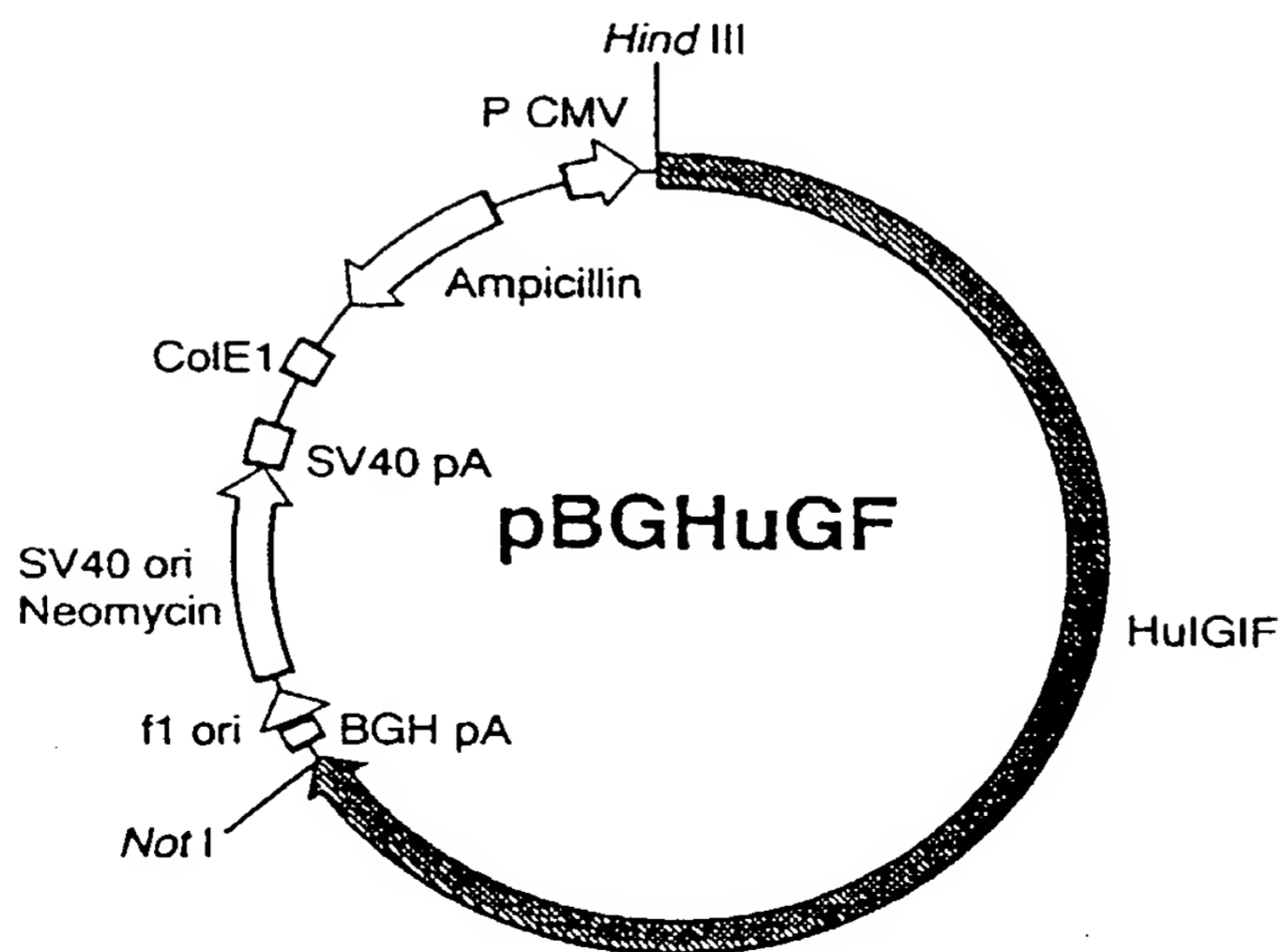


FIG. 2



**FIG. 3**



**FIG. 4**

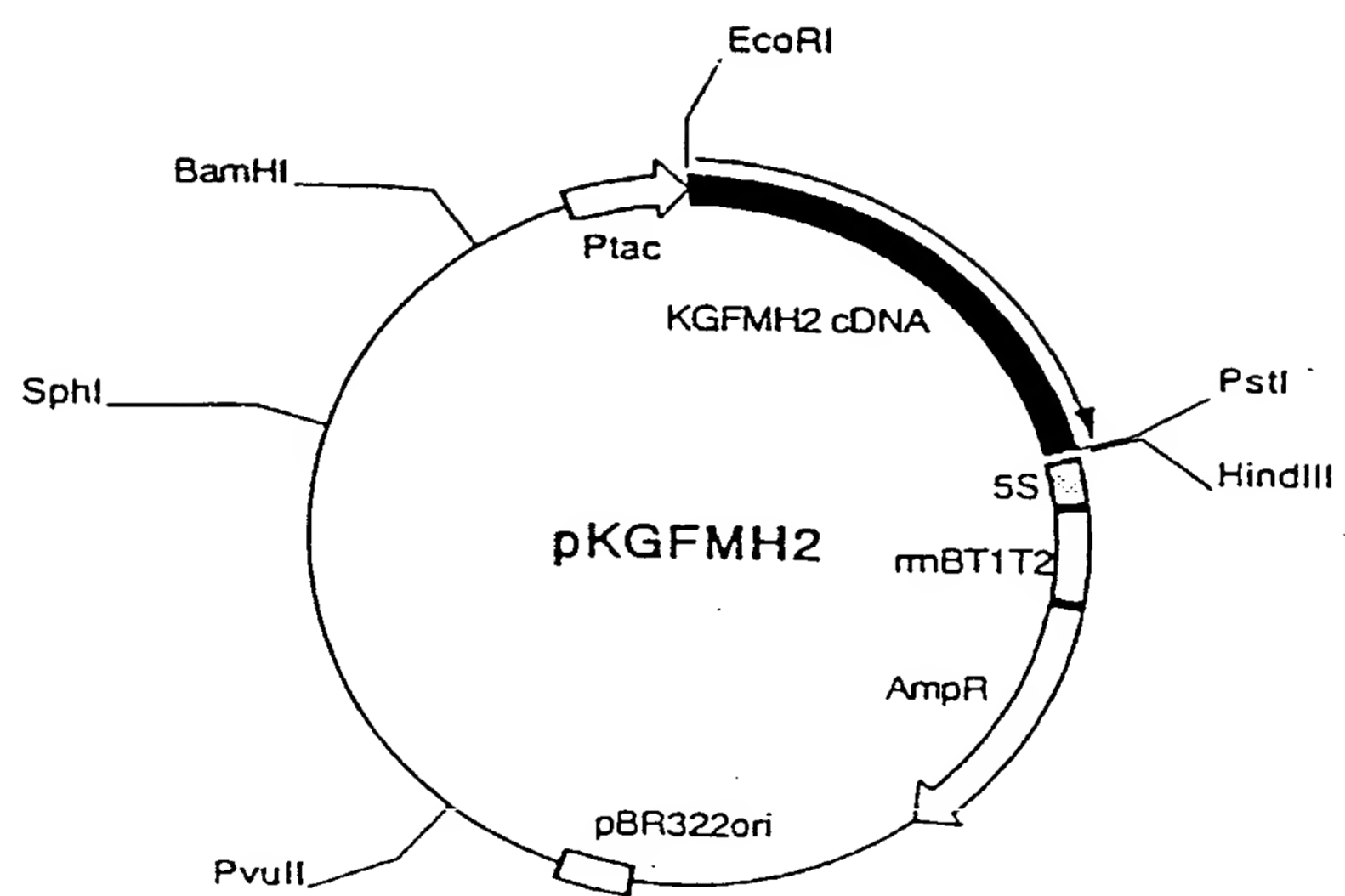


FIG. 5